THE DURIAN THEORY EXTENDED — III. PACHYCAULY AND MEGASPERMY — CONCLUSION

E. J. H. CORNER
Botany School, Cambridge, England

Pachycaul and Leptocaul

Whether the flower is examined, as by Arber and Parkin (1907), or the fruit and the leaf (Corner, 1954), the ancestral form appears as a bulkier construction with more extensive apical growth. The same contrast arises in the study of tree-shapes. On the one hand, there are tall, highly branched trees with dorsiventral branch-systems ending in slender twigs with the leaves set in two rows; on the other, there are short, unbranched or sparingly branched trees with large leaves spirally arranged, as in a rosette, on a stout ascending stem. The first I have called *leptocaul* to indicate the slender primary construction with small apex; the second I called the *pachycaul* with large apex and massive primary construction, reflected in the large pith or the scattered vascular bundles. Many families and, even, genera possess both states. Therefore, whatever the evolutionary connection between the states, they are polyphyletic in the sense of having been evolved independently in different families and, even, genera. What is more, the evolutionary aspect is visible, not imaginary, because the variety exists today in the tropical forests.

A priori the leptocaul is the more advanced. It is more ramified and, therefore, its xylem-connections are more complicated. Its lateral shoots become dorsiventral. The primitive spiral phyllotaxis may persist on the main stem, but it becomes distichous on the dorsiventral shoots. The internodal elongation may vary, and short shoots may be developed. These points indicate more complicated construction, and the very height of the leptocaul indicates more complicated engineering: it can be seen, indeed, in the deciduous habit which rarely, if ever, occurs in the pachycaul. In this form, however, the branch is merely a replica of the main stem. The applanate, dorsiventral shoot, so dissimilar from the erect main shoot as even to bear different leaf-forms (as in the Phyllantheae and in species of *Ficus*), is clearly derivative as the branch that most successfully spreads the crown of the tree outwards: the leptocaul presents the division of labour into upwardly growing stems and outwardly growing branches, whereas the pachycaul is the original compromise. Leptocaul and pachycaul evolution becomes the evolution of the tropical forest. The taller the tree, the more advanced is its construction in the upward struggle for light.

Genera such as *Cassia*, *Elaeocarpus*, *Eugenia*, *Symplocos*, *Litsea* and *Sapium* show the transition from the radially constructed tree with spiral or decussate leaves on ascending twigs to that with applanate foliage; and they show that in the transition the primary construction of the twig is more leptocaul than that of the erect leader. The more slender twig is the more advanced. Dormer (1953) has shown that in *Cassia* the applanate foliage is a systematic character marking evolution within the genus. This distinction in tree-form occurs also in pairs of genera, such as *Salix* (with mainly applanate foliage in its tree-forms) and *Populus* with spiral foliage, and similarly *Fagus-Quercus* or *Betula-Alnus*, as well as at higher levels as Amherstieae-Eucaesalpinieae and Magnoliaceae-Annonaceae. In other genera, such as *Sterculia*, *Cola*,

Hibiscus, *Solanum*, *Artocarpus* and *Ficus* there are distinctions among the tree-forms with spiral, ascending foliage. The species with thickest primary construction, producing twigs about 10 mm. thick bear more or less compound leaves with much less internodal elongation than the species with slender twigs, 2-5 mm. thick, and smaller or simple leaves. Then, in these same genera, the unbranched saplings of the more pachycaul species commonly bear still larger and more compound foliage on even stouter primary stems. That is to say, among the species of many widely different genera of trees there is a transition from megaphyllous pachycaul to microphyllous leptocaul, and this transition can be seen, as ontogeny recapitulating phylogeny, when the sapling grows up.

In general, six effects accompany the transition from pachycauly to leptocauly:

1. The tree heightens.
2. The primary construction of the twig diminishes.
3. The ramification increases.
4. The size and complexity of the leaf decreases.
5. The phyllotaxis decreases.
6. The internodes lengthen.

Thus, a simple rosette-form of tree develops into a lofty umbrageous crown. And if this is not the manner in which the tree-form has evolved in the flowering plant, then go into the tropical forest and discover the truth !

The biggest trees of the forest are, for their families, leptocaul, e.g. *Sequoia*, *Pseudotsuga*, *Abies*, *Ulmus*, *Fagus*, *Eucalyptus*, *Bertholetia* (Lecythidaceae), *Dryobalanops* (Dipterocarpaceae), and *Mora*, *Dinisia* and *Koompassia* in Leguminosae. At the other extreme, the largest compound leaves are borne on the most massive primary stems of relatively short and little branched trees, such as *Aglaia*, *Carica*, *Ricinus*, *Schefflera* and, of course, palms, cycads and tree-ferns.

Pachycaul and Leptocaul in the Forest

The seedling of a pachycaul will gradually build up a massive primary stem and a large rosette of compound leaves. It will heighten mainly by the slow production of more leaves, but their very size, longevity and close arrangement will create a smothering effect on other seedlings. The pachycaul occupies much space for little gain in height, and even the old trees, like long-stalked umbrellas, as I have seen the old cycads in the undergrowth of the Malayan forests, are too shady for much to grow underneath. The seedling of a leptocaul, on the other hand, quickly develops a slender stem, heightened easily by internodal elongation, with small leaves scattered along it: it occupies little space for much height. So, other things being equal, the leptocaul seedling must be more numerous and better fitted for the upward struggle for light. The leptocaul, therefore, will have a higher evolutionary rate and an ecological dominance. For temperate climates, an apt comparison would be the thistle and the nettle. Internodal elongation is structurally impossible in large stems with multilacunar leaves in high phyllotaxis.

Consider a cylindric, palm-stem 30 × 20 cm. When transformed into a cylindrical leptocaul, 2 mm. in diameter, the same volume would give a length of 300 m. For the same amount of assimilation, or initial food supply in the seed, the leptocaul will rapidly overtop the pachycaul. This is the liane-effect. On the other hand, if a pachycaul develops a leaf 2·5 m. long and occupies a horizontal area of 25 sq. m., and a leptocaul develops a leaf 10 cm. long and overshadows an area of 0·04 sq. m., the packing could be about 600 leptocaul plants to one pachycaul. Thus the transformation of pachycaul into leptocaul brings about the characteristics of the tropical rain-forest, which are height, great variety of species and complex undergrowth. As the tree-form changes, there is physiological evolution: footholds increase and new environments appear in the rising forest.

Pachycaul Monocotyledons

Palmae — The palm-seedling builds up a massive stem-apex on the embryonic axis. On reaching a specific size, the crown is lifted on the columnar trunk without secondary thickening and, in

many cases, without internodal elongation. Branching does not occur, except for axillary inflorescences or basal soboliferous shoots. There is no evidence that the palm has progressed further in tree-evolution. The exceptional branching of *Hyphaene* and *Chrysalidocarpus* may suggest that the monocaulous habit is secondary through dominance of the exaggerated stem-apex, but, unlike dicotyledonous and other monocotyledonous stems, removal of the apical bud generally leads to the death of the whole palm-stem, and very rarely to axillary branching. I am inclined, therefore, to regard the tall, unbranching stems of such palms as *Roystonea*, *Caryota*, *Borassus* and *Corypha*, as lacking vegetative axillary shoots and, thus, primarily monocaulous. Other palms, both monocaulous and soboliferous, have been reduced and flower with physiological precocity in sapling and, even, seedling stages of small stature, e.g. *Pinanga* and *Geonoma*. Yet others, through decreasing phyllotaxis, determined probably by decreasing size of the stem-apex, have developed internodal elongation to produce the rattan. The history of palm-stem evolution is, indeed, to be read even now in *Calamus*.

PANDANACEAE — In habit the pandans are nearer to the arborescent Liliaceae and develop in the same way true, if lax, axillary branching. Even monocaulous pandans sprout axillary shoots when the stem-apex is removed. Hence the monocaulous species may be secondarily unbranched, as is certainly the case with the dwarf, herbaceous, and precociously maturing species barely half a metre high. *Freycinetia* is the more leptocaul root-climber, such as *Carludovica* in Cyclanthaceae.

SCITAMINEAE — This large series derives its peculiarities from the arborescent Strelitziaceae, which have the pachycaul stem supporting the huge leaf and the more primitive flower and fruit. Distichous construction permits internodal extension in Strelitziaceae, but it is lacking from the Musaceae with the primitive spiral construction. Even the herbaceous families have essentially pachycaul rhizomes, though their aerial stems of limited growth rapidly become leptocaul and attenuate, even to the state of non-flowering: special flowering shoots then develop from the rhizome as a peculiar instance of cauliflory.

ARALES — *Philodendron*, *Anthurium*, *Dieffenbachia* and *Amorphophallus* show not merely the reduction and simplification of the compound aroid leaf (Corner, 1954), but, in conformity, decrease in the stem-size. *Alocasia*, with erect stem, is truly pachycaul, and so is *Montrichardia*, the stem of which tapers off at *c.* 6 m., though the sympodial construction of the aroid may be a complication. The corm is the young pachycaul stem, geophyllous and seasonal in *Arum*, and monophyllous in *Amorphophallus*, in which the leaf-size is evidently exaggerated as in the larger palms. The corm introduces again the phenomenon of precocity in maturing, which on aroid lines has led to the very reduced Lemnaceae. The conclusion seems inevitable that a stout pachycaul stem with large compound leaf was the ancestral growth form of the Arales, and that it has passed on mainly to epiphytic, geophytic and hydrophytic derivatives as the forest evolved.

LILIIFLORAE — *Dracaena*, *Cordyline*, *Xanthorrhoea*, *Fourcroya*, *Yucca* and *Aloe* are the pachycaul, arborescent genera with the simple phyllodic leaf and little internodal elongation, as in *Pandanus*, but with varying amount of secondary thickening. It is customary to regard them as derived from the herbaceous and geophilous members of the order and to link them with the palms as a further upgrade. But the series can be read in either direction and I see no compelling evidence in favour of the upgrade. On the other hand, there is clear evidence in the rain-forest genus *Dracaena* of reduction from the tree-form to the monocaulous herbaceous condition as in *Pandanus* and the dwarf palms, for there are all gradations from large, much ramified trees to little herbaceous species barely a foot high. On comparison with the palm and the aroid, the geophytic Liliiflorae are derivative from the arborescent, having little satisfactory place in the forest. Thus the rosette-form, as *Agave*, as well as the corm, bulb and rhizome, appear as the herbaceous derivatives of

the pachycaul selected for extra-tropical stations.

Bromeliaceae — The rosette-form, analogous with *Agave*, is shown by the monocarpic *Puya*, capsular and ornithophilous as the Strelitziaceae. On taxonomic grounds it is regarded as the most primitive genus of the family (Smith, 1934): it is also the pachycaul.

Juncaceae — Possibly *Prionium* is the pachycaul in this mainly leptocaul and extra-tropical family. The more removed a family is from the tropical rain-forest, the less clear is the pachycaul connection.

Gramineae — In many reproductive and vegetative features the bamboo is antecedent to the grass (Arber, 1934). The large bud and massive culm without secondary thickening show that it is pachycaul: the festucoid grass is leptocaul. But the bamboo differs from all other pachycaul monocotyledons, that I have mentioned, in the manner in which the large bud is built up: it is not formed on the embryonic axis, but is developed sympodially on successively enlarged soboliferous branches. Thus the grass, representing the precociously matured leptocaul phase of the young bamboo, brings a new feature into the vegetable world which we cultivate to perfection in the lawn. Further the bamboo has distichous phyllotaxis, which is the arrangement best suited to internodal extension because of the minimum overlapping of the leaf-traces. This feature, coupled with the siliceous strengthening of the cell-walls, has turned the relatively short, solid-stemmed ancestral culm into the telescopic tube rapidly projected on the capital of preceding culms and petering out on reaching its physiological limit in many leptocaul, photosynthetic ramifications. It is as novel a forest-type as the grass in the open, though the calamites must have been analogous. It is, however, the pachycaul that links the arborescent and the herbaceous in this large family, as it does in others. *Zea*, *Saccharum*, *Sorghum* and other panicoid grasses, with solid stems built on the *embryonic* axis, stand out then with exceptional interest as nearest to the protogramineous form.

Cyperaceae — I do not understand this advanced family, but *Cyperus papyrus* and the larger species of the closely allied *Scirpus* appear to me comparable with the solid-stemmed and pachycaul panicoid grasses. Holttum (1948) emphasizes the resemblance between *Mapania* or *Scirpodendron* and *Pandanus*, which is another way of indicating the pachycaul as the primitive forbear.

Conclusion — In Palmae, Pandanaceae, Scitamineae, Arales, Liliiflorae, Juncaceae, Bromeliaceae, Gramineae and Cyperaceae there is evidence for derivation from a pachycaul ancestor. It follows that the evolution of the orders of monocotyledons, if not of the families, took place in the pachycaul stage which, excepting the Palmaceae and Pandanaceae, is largely extinct or modified into the herbaceous form with pachycaul storage organ. Hence it is understandable that there should now be so little connection between the main orders of monocotyledon and such great differences among their herbaceous derivatives, that they can mostly be recognized from vegetative characters.

The Pachycaul Apex

A massive primary construction, supporting large compound leaves in high phyllotaxis, implies a massive meristem. There is now considerable information about the size of the growing tip of the stem, that is the apical dome or disc between the incipient leaf-primordia. Thus,

Cycadaceae: 0·6-3 mm./wide (Johnson, 1951)

Palmae: 0·5-1 mm./wide (Wardlaw, 1953; Corner, ined.)

Cactaceae: 0·7 mm./wide (Wardlaw, 1953)

Nuphar: 0·6 mm./wide (Wardlaw, 1953)

Angiosperms generally: 0·02-0·3 mm. wide; Rosaceae, 0·03-0·1 mm./wide (Rouffa and Gunckel, 1951)

Coniferae: 0·06-0·2 mm./wide (Johnson, 1951).

These measures are not, however, a true measure of the size of the apical meristem. Behind and around the extreme growing point, to which these measures refer, there is a mass of meristematic tissue which builds up the pith, cortex and vascular bundles. It is this sub-apical mass which

is so conspicuous in pachycaul monocotyledons and which makes a simple linear measure of the tip so inadequate. Thus, in *Elaeis* (Palmae), the tip is only 0·5 mm. wide (Wardlaw, 1953), but the whole apical meristem is to be measured in cubic centimetres. Leptocauly, therefore, can arise in two ways, by reduction in size of the growing tip or of the sub-apical mass. Most studies of apices have dealt with leptocaul stems, which show simplifications connected with microphylly and, also, an exact arrangement of tissues in few-celled layers. It is by no means clear to me that the pachycaul apex has any such exact definition into tunica and corpus. Indeed, when the apex expands to form the inflorescence or the flower, this very simplification into layers disappears. The disappearance has been held to be a distinction between vegetative and reproductive apices on a purely academic basis. To the durianologist the expanded floral apex is a reversion to pachycauly. Here, then, as so often, the study of the tropical plant is needed to orientate the studies on temperate plants.

With regard to the vascular supply, a single ring of vascular bundles is clearly inconsistent with so much ground-tissue. The so-called diffuse, or scattered, system of bundles permeating the ground-tissue and developed in great complexity in the sub-apical part of the meristem is the monocotyledonous solution, repeated to some extent in the cycads. It appears naturally as the primitive construction from which, at the leptocaul extreme in grasses, the single ring of bundles round the hollow pith is the configuration for small meristems. Here, again, the simplicity and precision of the monocotyledonous herb will not be understood until the very complex condition of the palm, bamboo, dracaena and banana has been unravelled. Then, I think, it will be better appreciated that the " anomalous secondary thickening " of the arborescent Liliaceae is a far from anomalous method of thickening a pachycaul construction.

Pachycaul Dicotyledons

It is fitting now to recall Worsdell's theory (1915, 1919) that the cortical and medullary vascular bundles of dicotyledons are relics of a primitive state possessing the diffuse, or scattered, vascular system. His argument based on a study of the Cucurbitaceae and Compositae seems conclusive. He attributed the construction, however, to an original geophilous habit. It would apply equally well to a pachycaul arborescent habit, and geophily is a specialization. The durian theory meets Worsdell's anatomical theory and supplies the plant-form.

The effect of the dicotyledonous cambium becomes apparent in the transition from pachycauly to leptocauly. A smaller, less dominant, growing point, with smaller leaves in simpler phyllotaxis, gives less expenditure in ground-tissue and permits both gain in height by internodal extension and canopy-construction by profuse branching. The secondary cambium thickens the slender, extensible ground-tissue. The single ring of vascular bundles and the precise cambium between phloem and xylem becomes the rule, accepted as the fundamental construction of the dicotyledon by orthodox botany built upon temperate floras, and any other construction becomes the " anomalous " as in monocotyledons. Here, the durian theory steps in and shows that these despised anomalous constructions are steps in the evolution of the orthodox, for which there is hitherto no explanation. The durian theory brings the " anomalous " thickening of lianes and beetroots into line as stages in the evolution of the perfect dicotyledonous construction, and shows where lianes and Centrospermae may have diverged in the course of angiosperm evolution. Orthodoxy has, in fact, almost stifled the study of lianes.

Now, where pachycauly still occurs among dicotyledons as a relic, there occur the cortical and medullary vascular bundles and the intraxylary phloem of Worsdell's theory. This theory meets, in fact, the durian theory so exactly that, if it had not been for the help of Dr. K. R. Sporne of the Cambridge Botany School, I would have blundered into proposing Worsdell's theory anew from the tropical end. In the following examples, which are far from complete, I have relied on the general massiveness of the primary stem

for evidence of pachycauly. The anatomical information is given by Metcalfe and Chalk (1950). The dimensions of the apical meristem are still to be discovered.

NYMPHAEACEAE — *Victoria amazonica* has a massive, erect stem, up to 60 cm. high (Decke, 1936), exactly as an underwater pachycaul. There are no internodes. The roots are adventitious from the leaf-bases. The vascular bundles are scattered. There is no cambium. There are no vessels. The megaphyllous leaves are thorny, but modified to the peltate, basipetal form. The thorny fruit has a vestigial aril. Yet, this wonderful plant figures in botany mainly for its horticultural value. It survives in the relic world of the manatee. Around the lakes where it grows rise the *Cecropia* trees on whose leaves the sloths feed. This is no accident, but a relic of ancient biology, as I will mention later. It behoves the botanist to consider this survival. Was it lack of vessels and of cambium that kept the Nymphaeaceae sub-aquatic? Were their ancestors part of the pre-mammalian pastures?

HALORHAGIDACEAE — *Gunnera* resembles *Victoria* in vegetative form, planted out of water. It, too, is "polystelic". *Myriophyllum*, and *Hippuris*, are the leptocaul derivatives, also aquatic, as the leptocaul Nymphaeaceae. But what is *Gunnera* in orthodox botany? A locus for *Nostoc*? It, too, is better known in horticulture.

CUCURBITACEAE — *Dendrosicyos*, of Socotra, is another extraordinary pachycaul. Leptocauly prevails in the family, though there is a tendency to produce fleshy green twigs, and it was from this family that Worsdell pronounced his theory.

CARICACEAE — No plant conveys to my mind the impression of the ancient pachycaul better than *Carica papaya*. Its leaves, however, are palmate and there is some internodal extension in the seedling and sapling. The vascular structure and secondary thickening, too, are modern. But why cannot it form a proper tree? Other species of the genus are less pachycaul, or even leptocaul with simple leaves and free branching. The family is allied with the Cucurbitaceae and may well show how the dicotyledonous ring of vascular bundles has arisen polyphyletically, as well as the normal cambium. I think that these features are as polyphyletic in various angiosperm lines as almost any other angiosperm progression, and that it is erroneous to regard secondary thickening as necessarily homologous throughout the class.

MORACEAE — *Dorstenia gigas*, also of Socotra, is clearly a pachycaul state of this rather central genus in the family. More or less pachycaul species or sapling stages occur in the tree-genera such as *Artocarpus*, *Cecropia*, *Ficus* and *Musanga*. These are, in fact, some of the most interesting genera in which to study the evolution of the tree-habit.

CACTACEAE — Wardlaw (1953) has shown that these plants may have large growing-points, as befits the obvious pachycaul construction. Leaflessness, succulence, sparse branching, and the desert-life have led to the orthodox view that the family is highly derivative and specialized. The spines, the peculiar aril, the massive primary construction, the cortical and medullary vascular bundles, and the meagre lignification indicate that the cactus is a xerophytic pachycaul with insufficient transpiration stream. Arborescence is, in fact, feeble among the Centrospermae where the Cactaceae seem to belong.

EUPHORBIACEAE — *Ricinus* is comparable with *Carica* and *Cecropia*, and it has medullary vascular bundles. *Macaranga* and *Mallotus* are two important genera showing the transition to leptocauly. The tree Euphorbias, with cortical vascular bundles, are comparable with the cacti. The family shows how far the inflorescence, flower, and fruit evolved while the plant-form was still at a primitive growth-level.

APOCYNACEAE — The thick primary stem and lack of internodes in *Pachypodium* are typically pachycaul. The leaves are reduced, but there are accessory vascular bundles. It is, surely, the cactoid derivative of the family, while *Tabernaemontana* is the arillate leptocaul tree of the rain-forest. *Adenia*, with many species, may well reveal the history of *Pachypodium* and the succulent Asclepiadaceae.

COMPOSITAE — On floral grounds *Senecio* has been regarded as a primitive genus for the family. Willis (1949), too, regards it as such on grounds of "age and area". In the tree Senecios it possesses some of the most striking pachycaul plants. *Espeletia* is comparable in the Andes. *Vernonia* has the pachycaul habit (*V. conferta*) as well as most other growth-forms. Worsdell found the best evidence for his theory in the family. The conclusion seems obvious that Compositae evolved as pachycaul plants, imperfect structurally and physiologically for the rising forest.

LOBELIACEAE — The tree Lobelias, with complicated system of vascular bundles, are comparable with the tree Senecios.

SOLANACEAE — The slender, leptocaul, and glabrous weeds of the *Solanum nigrum* alliance, as well as those of the *S. biflorum* and *S. tuberosum* alliances, can be related in habit through the multitudinous species of the genus to the stout-stemmed, little branched, megaphyllous, thorny and stellately hairy, shrubby species with 4-8 locular ovary, such as *S. quitoensis*. Similarly, the leptocaul trees of the *S. verbascifolium* alliance relate back. The genus parallels and illuminates the problem of the Compositae, even to the basipetal pinnate leaves. For Willis, too, *Solanum* is the primitive genus.

Other families with cortical and medullary vascular bundles show more or less pachycaul species or sapling stages, e.g. Araliaceae, Begoniaceae, Bombacaceae, Burseraceae, Campanulaceae, Meliaceae, Polygonaceae and Sterculiaceae. Many of these plants are competitive in the tropical forest and represent, probably, stages in the evolution of leptocaul canopy trees. More comparable with the relic pachycaul forms, dispersed in situations outside the forest, are the suffrutescent species of *Brassica, Cotyledon, Echium, Rheum* and *Statice*.

Lastly I will refer to two genera, so decimated by systematics that they are dropping out of general botany. They are *Rubus* and *Rosa*. The stout, thorny, sapling stems, with wide pith, tapering to the leptocaul flowering twigs, as in the bamboos, *Asparagus, Ruscus, Smilax* and other edible monocotyledons, are transitions from pachycauly to leptocauly in the more or less lianoid state. That is how the tropical botanist can look at Rosaceae.

CONCLUSION — Vestiges of pachycaul construction occur as massive primary construction and scattered vascular bundles in many dicotyledonous families. In most, leptocauly, based on the secondary thickening of the single ring of vascular bundles, predominates. But many genera, especially of the rain-forests, show transitions between the extremes. As with the monocotyledons, the main family differentiation of the dicotyledons must have occurred in the now largely extinct pachycaul stage. Thus, the tree-form, as well as the herb, or the simple leaf, or the inferior ovary, or the indehiscent fruit, or the secondary xylem, are polyphyletic and present their own peculiarities in natural, or phyletic, series of dicotyledons.

Megaphyte and Rosette-Trees

Parkin has kindly drawn my attention to Cotton's address (1944) on the tree Senecios and similar plants, which he called megaphytes. I regret that this contribution, published during the war, was unknown to me. Megaphytes are precisely what I have called pachycaul. Yet, I do not find the name so suitable. What I contrast as leptocaul should then be "microphytes", and this is hardly applicable to *Sebuoia* or *Eucalyptus*: mesophyte has, of course, another sense. Du Rietz (1931) used Warming's name "rosette-tree", and he cites "rosettenträger" of Reiter, "schopfbäume" of Drude, and "tuft-trees" of Warming as other attempts to describe these unfamiliar plants. To Malays they are known as "Ali's umbrella". I prefer contrasting adjectives, particularly when it is realized that there are both pachycaul and leptocaul herbs, shrubs, climbers, trees, epiphytes, geophytes, hydrophytes, mesophytes and xerophytes. Thus, *Amorphophallus* is a pachycaul geophyte, rather than a nannomegaphyte.

According to Cotton (1944), the dicotyledonous megaphytes "are found in the warm temperate zone or on the moun-

tains in the tropics". But, as I have shown, this is far from true. *Victoria* is more megaphytic than the tree Senecio, and typical megaphytes occur in the heart of the rain-forest, while the leptocaul tree may grow through a sapling stage which is as megaphytic. For convenience I give the following brief categories:

1. Unbranched or sparingly branched rain-forest megaphytes in some species of the following genera — Araliaceae (*Brassaia, Schefflera*, etc.), Connaraceae (*Jollydora*), Euphorbiaceae (*Agrostistachys*), Lecythidaceae (*Grias, Gustavia*), Meliaceae (*Aglaia*), Moraceae (*Ficus pseudopalma*), Sapindaceae (*Radlkofera, Talisia*), Simarubaceae (*Eurycoma*), Theophrastaceae (*Clavija*): to which may be added *Victoria* and *Carica papaya*.

2. More freely branched rain-forest megaphytes — Euphorbiaceae (*Ricinus, Macaranga, Mallotus*), Moraceae (*Cecropia, Ficus*) and numerous Araliaceae.

3. Leptocaul trees with more or less megaphytic or pachycaul saplings — Anacardiaceae (*Campnosperma*), Bignoniaceae (*Oroxylon, Pajanelia*), *Dillenia*, Flacourtiaceae (*Pangium*), Leguminosae (*Schizolobium*), Malvaceae (*Hibiscus*), Moraceae (*Artocarpus, Ficus, Musanga*), Papaveraceae (*Bocconia*), Polygonaceae (*Triplaris*), Sterculiaceae (*Sterculia, Scaphium, Cola*).

Certainly in the tropical rain-forest there are no well-defined systematic groups of dicotyledonous megaphyte, such as among the monocotyledons and the extra-rain-forest dicotyledons. The rain-forest families have evolved beyond this level, though it persists as a relic in various families. But there are, also, many transitions to the leptocaul trees that are not shown by the specialized xerophytic megaphytes. Indeed, the phenomenon of pachycauly-leptocauly repeats itself in the second degree in the rain-forest, for in several families and genera of monopodial trees with simple leaves the primary branch-systems function as the megaphylls, e.g. Annonaceae, Rhamnaceae, Rubiaceae and Phyllantheae (Euphorbiaceae). Thus, the fruit-tree *Cicca* (Phyllantheae) so curiously resembles the pinnate-leafed, sub-pachycaul tree *Averrhoa* (Oxalidaceae).

According to the durian theory, the pachycaul was the early tree-form of the smothering kind. It must once have formed the canopy of the angiosperm forest, even if this was riparian in taller gymnosperm forest. Therefore, the pachycaul was primarily photophilous. To survive in later forest, it must have become shade-loving, or transient as a colonizer of bare ground, or epiphytic, *or* xerophytic in deserts or on mountains and cliffs *in the warm regions*: for the large bud seems unadaptable to frost. On the other hand, it evolved into the pachycaul herb, geophilous and adaptable to frost and drought as well as shade. Thus I regard the tree Senecio as nearer the growth-form of the "proto-Compositae" than any other species of the genus: it survives outside the lofty forest.

There are gymnospermous parallels. *Welwitschia* is the desert relic, along with *Pachypodium*. *Gnetum* is the leptocaul liane. *Ephedra* is the microphyllous, xerophytic leptocaul. *Cycas* and *Microcycas* are rain-forest pachycauls, but other genera of the Cycadaceae are relic xerophytes. Conifers are microphyllous leptocauls. *Araucaria* in South America is a second order megaphyte.

Now that the systematic exploration of the tropics has progressed so far that new floras are being prepared, the field botanist will have time to pause and study in systematic order the growth-forms of the plants. Generic evaluation is needed, and there is an immense number of species which can be studied and which are now namable. Thus true comparisons will emerge with morphological precision and will lead to physiological investigation. The pachycaul habit is conspicuous in genera which, for some reason, have been unable to evolve the leptocaul. It may be a problem of transpiration-current, of wood-structure, of inefficient cambium, or, as Cotton suggested, of auxin-control. But there is abundant material wherein to search why tree- and herb-evolution has paused along so many different lines at so many slightly different levels. Thus, *Victoria* has gone underwater. *Carica* peters out as it heightens. Many palms cannot branch. The bamboo diffuses into leptocaulous distichy. But *Solanum, Ver-*

nonia and *Ficus* multiply into all sorts. This, to me, is the clarity of systematics.

Myrmecophily

Several of the plants I have mentioned are myrmecophilous. It cannot have escaped notice that the myrmecophilous plant, with ant-galleries, has a wide pith, self-excavating or excavated by the ants and, therefore, presents a *prima facie* case of pachycauly. As the septate pith, it is a property of the plant intermediate between the pachycaul and the leptocaul. Thus, *Cecropia*, *Macaranga*, *Archidendron* (de Wit, 1952), *Cordia* (Boraginaceae), and *Myrmecodia* and *Hydnophytum* (Rubiaceae) come to mind, and the myrmecophagous mammals which are also relics. A myrmecophilous panorama comes to view as an early stage in the elevation of the canopy. And these are the plants so often possessed of extra-floral nectaries, beloved of the ants. The subject has dropped out of modern botany, and this is where the durian theory helps. Not only have the vertebrates had less to eat as the angiosperm forest evolved, but the ants have been outstripped. Yet it is not too late for the biologist to enquire what has been the evolution of the epiphytic, xerophytic, spiny, pachycaul and myrmecophilous *Myrmecodia* with its bird-distributed seeds, like mistletoes. Is there a pachycaul mistletoe ? One thinks of the West Australian *Nuytsia* with its anomalous secondary thickening, scattered vascular bundles, numerous cotyledons and peculiar systematic relations.

" The unwritten chapter in natural selection is that of the evolution of environments " (Dewey, 1900).

Monocotyledons, Monocarpy and Monocauly

There is little that I can add to my conclusion that a palm-like phase preceded dicotyledonous forest. It may have been dicotyledonous in embryo, for I find no durianological evidence, but it was palm-like in megaphylly, pachycauly, scattered vascular bundles and spare branching. Out of it have come modern monocotyledons, some loftier but monocaulous, others more ramified with anomalous cambium, but most as herbaceous derivatives; and out of it came the dicotyledons, which built the greater forests where the higher animals evolved. One and two cotyledons suggest limiting cases, reduced from many, not whorled or decussate, but spirally arranged, as in primitive phyllotaxis on an embryonic axis; and, perhaps, this pro-angiosperm was polycotyledonous. I cannot help being impressed with the fact that the monocotyledon has stopped at the beginning of forest-evolution, which the dicotyledon carried on. Therefore, I argue, other monocotyledonous characters may be primitive. Parkin mentions the problem of the tap-root, developed so simply in the dicotyledon that one is apt not to consider its introduction. Nevertheless a tap-root, continuing as the shoot enlarges, means secondary thickening, for a " pachyrhiza " cannot be thrust into the soil as a pachycaul into the air, even though pandans have uncommonly stout roots and palms and *Victoria* to a lesser degree. If, then, as I have argued, the dicotyledonous cambium was not a primary feature of the pro-angiosperm, nor was the tap-root. My purpose has been to show that in the tropical forest the orthodox views of botany may not be applicable. We must be able to walk round the problem, though we are led to it from one direction.

The other feature which has impressed me is monocarpy, or the ending of vegetative growth by an infructescence. Gramineae, Cyperaceae, Juncaceae, Liliaceae, Bromeliaceae, Araceae, Scitamineae, Pandanaceae, and so many other families of monocotyledons have the character that the lateral inflorescence is remarkable. Monocarpy would seem the natural consequence of a shaded seedling growing up through a vegetative phase to a physiological threshold of reproduction at the fully illuminated surface of the forest. The monocotyledonous habit of suckering from the collar of the plant, as the neutral region between stem and root, would also seem the natural consequence of a residuum of food-reserves after the monocarpic fruiting, and the step to polycauly. Thus I argued that monocauly might be primitive, rather than a limiting

case. The durian theory supplies the large fruit that would drain the primitive stem, while living genera show the evolution of leptocauly and ramification. The greatest difficulty that I see is the evolution of the inflorescence, because I can find no relation between it and the evolution of the angiosperm tree, except to repeat that the evolution of the inflorescence and the flower to the level of modern orders, if not families, must have occurred at the pachycaul, pre-Cretaceous period. If not, then an entirely different theory of leaf, fruit and tree evolution must be elaborated, and I can find no evidence. The fact that the durian theory is an approach to the evolution of angiosperm forest without reference to the flower, while approaches to the evolution of the flower carry no reference to angiosperm forest, is a sign that the two processes have been independent.

The evolution of the embryo may be the link. Why should a pachycaul plant commence with a leptocaul embryo and then produce a much ramified, leptocaul inflorescence, as a palm or pandan? Why should it change into a leptocaul tree? The leptocaul appears to be a persistent seedling construction, that is thickened secondarily, and this seedling construction may have been related to floral simplification. The next step in durianology will be to consider the evolution of the inflorescence on the lines suggested by Parkin (1914), but with reference to pachycaulous plants and the other signs of the durian theory.

Megaspermy and Microspermy

As Parkin observes, there are all gradations in the size of the seed among and within the families of flowering plants. Nevertheless, many tropical and woody families are large-seeded compared with others that are small-seeded. Thus, purely in regard to the size of the seed, the following categories can be distinguished:

Megaspermous — Annonaceae, Bombacaceae, Burseraceae, Connaraceae, Dipterocarpaceae, Ebenaceae, Fagaceae, Guttiferae, Lauraceae, Lecythidaceae, Myristicaceae, Sapotaceae and Palmae.

Microspermous — Asclepiadaceae, Compositae, Ericaceae, Hypericaceae, Melastomaceae, Salicaceae, Urticaceae.

Megaspermous and microspermous — Apocynaceae, Euphorbiaceae, Leguminosae, Malvaceae, Moraceae, Myrtaceae, Papaveraceae, Rubiaceae, Rutaceae, Sapindaceae, Sterculiaceae, and Gramineae, Liliaceae and Scitamineae.

The definition of megaspermy will vary for each family. Thus, the small seeds of *Cassia* are almost as large as those of *Bocconia* (Papaveraceae), but not as small as those of some Moraceae and Rubiaceae. But there is another criterion more profound. In the small seeds of herbaceous plants such as Cruciferae, *Papaver*, *Oenothera* and *Solanum*, the cells of the two integuments merely enlarge after fertilization with little or no cell-division: the testa is, therefore, thin and small. In contrast, in the large seeds of the megaspermous families listed above, there is extensive cell-division to form a large and complicated testa, the structure of which is an important, though neglected, feature of the angiosperm. This massive testa provides a much better criterion for the distinction into mega- and microspermy; it puts the Leguminosae entirely into the megaspermous category. There are, nevertheless, as in all biological distinctions, intermediates.

Arillate seeds are generally large through extensive cell-division. Therefore, I conclude that loss of this cell-division, as well as the loss of the aril, is derivative and indicates that precocity which marks the evolution of the flowering plant towards its herbaceous destiny. Arillate seeds, too, like most large seeds, germinate directly. Dormancy, therefore, appears advanced, as one would expect from a physiological specialization. Largeness and non-dormancy fit the rain-forest habitat, as the primitive association of the flowering plant. Smallness, lack of aril and dormancy mark the advanced seed, which pioneers the new associations. It is not the result of extra-tropical conditions, but a tropical syndrome selected for the new habitats. All three characters have been evolved under tropical conditions in many different families and it behoves one to enquire into their occurrence in the tropi-

cal forest. To suppose that the small seed, which ripens quickly with minimum somatic growth and then is moved about dormant, should be primitive is to mistake the simplicity of efficiency. The problem of the small seed in the rain-forest is well explained in the following quotation about quinine-trees (*Cinchona*):

"With the abundance of seeds yielded by the cinchonas, one would naturally expect young plants to spring up in great numbers, but such is not the case; the light-winged seeds mostly fall upon and adhere to the evermoist foliage, where they quickly germinate and decay; or, if perchance they fall to the ground, it is exceedingly difficult to gain a rooting, as the soil is covered to a depth of ten to twenty inches with loose, decaying leaves" (Wellcome, 1880).

Fig plants, which are microspermous, develop usually in more or less bare places of the forest-soil, as by rivers, shores, rocky outcrops and landslips, or as epiphytes. The megaspermous *Artocarpus*, in contrast, is essentially a forest-tree. *Rhododendron*, as *Cinchona*, is a lower temperature counterpart of *Ficus*. The microspermous orchid contrasts with the megaspermous palm and reveals its precocity by flowering before the development of the ovules.

Parkin's suggestion that the mechanism of dormancy may be connected with the metabolism and food-store of the seed, oily seeds being less resistant, is most interesting and emphasizes the need for a comparative physiology on the lines of systematic morphology.

Conclusion

The durian theory places tropical forest in evolutionary perspective. It is based on the following arguments derived from genera of tropical, arborescent dicotyledons and monocotyledons:

1. Modern fruits can be traced back to the fleshy red capsule or follicle with many large, arillate, non-dormant seeds.

2. The glabrous epidermis can be traced back to the glandular, peltate-scaled and spinous.

3. The phyllodic leaf can be traced to the simple and pinnately veined leaf, which can be traced back to the large, several times pinnate leaf.

4. The dorsiventral, or applanate, spray of foliage can be traced back to the stouter ascending twig with spirally arranged leaves.

5. This ascending twig can be traced back to still stouter twigs with larger leaves borne on sparingly branched or unbranched plants, styled the pachycaul.

6. The characteristic of the pachycaul is a large apical meristem (not merely a large growing-point), scattered vascular bundles associated with megaphylly, sparse branching, little or no internodal elongation, and low stature.

7. The pachycaul apex could have accommodated the very large fruit postulated by the durian theory.

The theory envisages the gradual evolution of the modern tropical forest in height, complexity, leaf and fruit from the short, umbrella-shaped, smothering, arillate pachycaul in response to the upward struggle for light. As the large, parenchymatous leaves, fruits and buds were transformed into the small, simple leaves, dry fruits and leptocaul twigs, there was less food for animals, but a much greater variety of arboreal habitats. Thus, the zoology of the tropical forest evolves with the botany.

Palaeobotany forces the conclusion that the modern tropical forest had evolved by the lower Cretaceous period. Its evolution from the pachycaul stage, when floral evolution had already happened, must have been Jurassic or earlier. What, then, of the animals? There is no fossil trace. Text-books indicate that birds and mammals were the products of the Cretaceous period. Presumably the Jurassic angiosperm forest contained pre-avian and pre-mammalian forms, but the great hiatus of palaeobotany becomes the great hiatus of palaeontology. The durianologist will realize that modern forest animals are merely the specialized end-products of a far greater variety, though the invertebrate fauna probably expanded *pari passu* with the forest.

The omission of the tropical forest from evolutionary thought has resulted in short-sighted and erroneous views of both flowering plant and vertebrate evolution.

In attempting to meet most, if not all, of Parkin's criticisms, I have tried to show

that the big genera of the tropics provide the keys to many of the problems. Fuller treatment must await the fuller study of these genera. Here is a great opportunity for the field botanist of the tropics, now that there is an adequate systematic background.

Summary

It is argued that the pachycaul, or megaphytic, plant-form was primitive. The conversion of this to the leptocaul is considered in its effect on heightening the forest and increasing its individual content as a new environment.

Worsdell's theory, postulating the derivation of the simple dicotyledonous vascular ring or cylinder from a state with scattered vascular bundles, is revived and identified with the pachycaul stage in evolution.

Myrmecophily and " edentate zoology " may be identified with the transition from pachycauly to leptocauly.

Seed-size is reconsidered with the conclusion that megaspermy may be identified rather with copious cell-divisions in the outer integument than with absolute size.

The lack of fossil remains of the early angiosperm forest must also be reflected in the zoological record.

Literature Cited

Arber, A. 1934. " The Gramineae." Cambridge.

Arber, E. A. & Parkin, J. 1907. On the origin of angiosperms. J. Linn. Soc. (Bot.) **38**: 29-80.

Corner, E. J. H. 1954. The durian theory extended—II. Phytomorphology **4**: 152-65.

Cotton, A. D. 1944. The megaphytic habit in the tree Senecios and other genera. Proc. Linn. Soc. London Session 156: 158-168.

Decke, J. S. 1936. " Aspectos biologicos da Flora Brasileira." São Leopoldo (Brazil). p. 47.

Dewey, J. 1900. Evolution and ethics. The Monist **8** (reprinted in Sci. Mon. N.Y. **78**: 57-66, 1954).

Dormer, K. J. 1953. A study of phyllotaxis in the genus *Cassia*. New Phytol. **52**: 313-315.

Du Rietz, G. E. 1931. Life-forms of terrestrial flowering plants. Acta phytogeogr. Suec. **3**: 55-56.

Herbert, D. A. 1918. The Western Australian Christmas tree. J. Roy. Soc. W. Aust. **5**: 72-88.

Holttum, R. E. 1948. The spikelet in Cyperaceae. Bot. Rev. **14**: 525-542.

Johnson, M. A. 1951. The shoot-apex in gymnosperms. Phytomorphology **1**: 188-204.

Metcalfe, C. R. & Chalk, L. 1950. " Anatomy of the Dicotyledons." (Vols. I & II). Oxford.

Parkin, J. 1914. The evolution of the inflorescence. J. Linn. Soc. (Bot.) **42**: 511-563.

Rouffa, A. S. & Gunckel, J. E. 1951. A comparative study of vegetative shoot-apices in the Rosaceae. American J. Bot. **38**: 290-300.

Smith, L. B. 1934. Geographical evidence on the lines of evolution in the Bromeliaceae. Bot. Jb. **66**: 446-468.

Wardlaw, C. W. 1953. Comparative observations on the shoot-apices of vascular plants. New Phytol. **52**: 195-209.

Wellcome, H. S. 1880. The Cinchona forests of South America. (Reprinted in Sci. Mon. N.Y. **71**, 205-208, 1950.)

Willis, J. C. 1949. " The Birth and Spread of Plants." Geneva.

Worsdell, W. C. 1915. The origin and meaning of medullary (intraxylary) phloem in the stem of dicotyledons. I. Cucurbitaceae. Ann. Bot. London **29**: 567-590.

— 1919. Ibid.— II. Compositae. Ann. Bot. London **33**: 421-458.

Wit, H. C. de. 1952. A revision of the genus *Archidendron* F. Muell. (Mimosaceae). Reinwardtia **2**: 69-96.

A NOTE ON NUCLEAR ENDOSPERM AS A PRIMITIVE CHARACTER AMONG DICOTYLEDONS[1]

K. R. SPORNE
Botany School, Cambridge University, England

The two main types of endosperm development, nuclear and cellular, were first recognized by Strasburger (1880) and subsequently an intermediate type, the helobial, was described. In the nuclear type, the primary endosperm nucleus undergoes one or more divisions without any cell wall being laid down. The daughter nuclei thus remain free, at least in the early stages, though subsequently they may become separated by cell walls. In the cellular type, it is usual for each nuclear division to be accompanied by cell wall formation, while in the helobial type, although the first nuclear division is accompanied by the formation of a cell wall dividing the embryo sac into two compartments, subsequent divisions are usually without cell walls. Ever since the discovery of these main types of endosperm, there have been discussions concerning their evolution. Thus, on the one hand, since it sometimes happens during nuclear endosperm formation that rudimentary cell plates appear, these have been interpreted by some as relics of a cellular ancestry (Coulter & Chamberlain, 1903; Schürhoff, 1926; and Glišić, 1928). Such workers, therefore, regard the nuclear condition as advanced and the cellular primitive. On the other hand, many botanists have followed Schnarf (1929) in holding the contrary view that nuclear endosperm is primitive, and base their belief on the observation that free nuclear divisions occur in the female gametophyte of gymnosperms. Maheshwari (1950) has, however, remarked that we still do not really know which way endosperm evolution has gone.

Some years ago (Sporne, 1948, 1949) the results were published of a statistical analysis of floral and vegetative characters among dicotyledon families, from which it was clear that some characters show a high degree of correlation. It was argued that, of the several possible explanations, the most probable one is that such characters are primitive and have survived together from ancient times. This view was to some extent upheld by the fossil record, and twelve characters were thereby listed as being primitive. More recently (Sporne, 1954) in a reply to criticisms by Stebbins (1951), I remarked that I had discovered further characters which could be added to the list. One of these is nuclear endosperm. I did not, however, state the evidence on which this claim was based, and it is my purpose to do so here.

The evidence is presented in Table 1, where it will be seen that nuclear endosperm (character "X") shows a high degree of correlation with seven floral or vegetative characters ("Y"). (Note that, for the purposes of calculation, helobial endosperm is here included with nuclear.) For each separate calculation, "n" represents the number of families about which information is available. In most cases this is 161, for this is the number of Engler's families (Engler & Diels, 1936) about which Schnarf (1931) gives endosperm information. The number is less than this in the case of characters 4 and 5 for clearly, in considering whether petals are free or united, those families without petals must be excluded, and in considering whether the stamens are more numer-

1. The substance of this communication was read before Section 8 of the Eighth International Botanical Congress in Paris, 1954.

TABLE 1

"Y"	n	x	y	m	m_1	χ^2	
Woody habit	161	111	119	95	82	25.5	+
Secretory Cells	161	111	59	53	40.4	19.7	+
Stipules present	159	110	66	61	46.3	26.4	+
Petals free	122	85	85	69	59.2	17.6	+
Stamens not meiomerous	154	107	134	105	93	19.9	+
Two integuments	161	111	97	87	67	48.6	+
Integument bundles	161	111	45	41	31	28.3	+
Fossil Record.	161	111	57	48	39.3	9.5	+

ous than the perianth members, those families without a perianth should be excluded. Similar considerations explain the variations in "x" (the number of families with character X = nuclear endosperm). "y" is the number of families with character Y and "m" is the number with both X and Y. "m_1" is the expected number of families with both characters, assuming that they are distributed at random ($m_1 = x.y/n$). In all cases, m and m_1 are different, showing that the characters are not distributed at random, and the figures calculated for χ^2 enable one to test whether these differences are significant. In each case, the value of χ^2 corresponds to a probability of much more than a thousand to one that the difference is significant. Furthermore, m being greater than m_1 indicates a positive correlation in each case.

The last line of Table 1 shows the occurrence of nuclear endosperm among families believed to have existed in pre-Pleistocene times, by reason of the identification of their fruits or seeds (Reid & Chandler, 1933) or of their woody stems (Edwards, 1931). Nuclear endosperm occurs in 48 of these 57 families instead of the calculated 39·3 families. The value of χ^2 here corresponds to a probability of nearly a thousand to one, which indicates that nuclear endosperm is more common among these families than in the whole flora of the world today.

The seven characters used are almost self-explanatory, but a few remarks about them should nevertheless be made. The heading " woody habit " includes all families in which at least some species are trees or shrubs, and under " stamens not meiomerous " are listed those families in which the number of stamens is equal to or greater than the number of perianth members. The families with " secretory cells " are those listed as such by Metcalfe and Chalk (1950) (and replace those listed in my previous publications as having " glandular leaves "). Families in which at least some species have vascular bundles in the integuments of the ovules are listed under " integument bundles ".

Not only are these seven characters correlated with nuclear endosperm, they are also correlated with each other to a high degree, as is shown in Table 2, where information already published elsewhere has been extracted specially to illustrate this point. The sign "+" indicates a positive correlation whose significance is greater than fifty to one and the sign "+" indicates a positive correlation which is less significant than this. The complete absence of negative correlations is most striking and so is the fact that all eight

TABLE 2

	Woody habit	Secretory cells	Stipules present	Petals free	Stamens not meiomerous	Two integuments	Integument bundles	Nuclear endosperm		Fossil record
Nuclear endosperm	+	+	+	+	+	+	+			+
Integument bundles	+	+	-+-	-+-	+	-+-				+
Two integuments	+	+	+	+	+					+
Stamens not meiomerous	+	+	+	+						+
Petals free	+	+	+							+
Stipules present	-+-	+								+
Secretory cells	+									+
Woody habit										

characters are more abundant in pre-Pleistocene families than among the families of the world flora. It is inconceivable that all the eight characters should be genetically linked or that they could all be interdependent in any functional manner, and the only reasonable explanation is that they are primitive characters. However, it must be emphasized that the evidence regarding endosperm is not yet conclusive, since it is based on only 161 out of the total of 259 families recognized by Engler and Diels. When details of endosperm development become more fully known, all the calculations must be repeated and the evidence re-assessed. Nevertheless, in spite of this limitation, the evidence is already very strong that nuclear endosperm is more primitive than cellular. It is so strong that I suggested (Sporne, 1954) the inclusion of nuclear endosperm in the list of characters to be used for assessing the relative advancement of dicotyledon families.

Summary

Among dicotyledon families, nuclear endosperm shows highly significant positive correlations with: (1) woody habit, (2) presence of secretory cells, (3) stipules, (4) petals free, (5) number of stamens equal to or more than the number of perianth members, (6) ovules with two integuments, (7) vascular bundles in the integuments. These characters are themselves correlated to a high degree with each other, which suggests that they, together with nuclear endosperm, are primitive characters. This is supported by an analysis of families believed to have been in existence before the Pleistocene period. Endosperm is, therefore, believed to have evolved from nuclear to cellular.

Literature Cited

COULTER, J. M. & CHAMBERLAIN, C. J. 1903. "Morphology of Angiosperms." New York.
EDWARDS, W. N. 1931. Fossilium catalogus. II. Plantae. Pars **17**: Dicotyledonae (Ligna). Berlin.
ENGLER, A. & DIELS, L. 1936. "Syllabus der Pflanzenfamilien." Berlin.
GLIŠIĆ, Lj. M. 1928. Development of the female gametophyte and endosperm in *Haberlea rhodopensis* (Friv.). Bull. Inst. Jard. Bot. Univ. Belgrade **1**: 1-13.
MAHESHWARI, P. 1950. "An Introduction to the Embryology of Angiosperms." New York.
METCALFE, C. R. & CHALK, L. 1950. "Anatomy of dicotyledons, leaves, stem and wood in relation to taxonomy, with notes on economic uses." Oxford.
REID, E. M. & CHANDLER, M. E. J. 1933. "The London Clay Flora." Brit. Mus. (Nat. Hist.)
SCHNARF, K. 1931. "Vergleichende Embryologie der Angiospermen." Berlin.
SCHÜRHOFF, P. N. 1926. "Die Zytologie der Blütenpflanzen." Stuttgart.
SPORNE, K. R. 1948. Correlation and classification in dicotyledons. Proc. Linn. Soc. London 160th Session: 40-47.
— 1949. A new approach to the problem of the primitive flower. New Phytol. **48**: 259-276.
— 1954. Statistics and the evolution of dicotyledons. Evolution **8**: 55-64.
STEBBINS, G. L. Jr. 1951. Natural selection and the differentiation of angiosperm families. Evolution **5**: 299-324.
STRASBURGER, E. 1880. "Zellbildung und Zelltheilung." Jena.

STUDIES IN FLORAL ANATOMY — VII. ON PLACENTATION IN THE CUCURBITACEAE

V. PURI
Meerut College, Meerut, India

Introduction

Notwithstanding the publication of numerous papers on the Cucurbitaceae during the last one hundred years or so, our notions about the morphology and taxonomy of this interesting group remain rather vague. The present author has been interested in the family for more than a decade now[1]. But owing to the difficulty of getting suitable material of certain species which appear to be of special consequence, the work has not progressed as well as it should have. However, some information has been gathered which appears to clear up the problem of placentation in this family.

Historical

Reference to placentation in *Cucumis sativus* is made by Nehemiah Grew (1682) in his famous book, "The Anatomy of Plants . . .". He wrote: "the middle parenchyma is divided into three columns which stand triangularly, having each of them a triangular figure. " . . " These columns are, as it were, beds on which seeds grow."

Later on, Gaertner (1791) studied the more obvious structural features of the

1. The work on Cucurbitaceae was started by the author in 1939. In 1943 it was passed on, for completion, to Mr. (now Dr.) M. L. Gattani, who joined the Meerut College as a colleague. Mr. Gattani prepared microtome slides of some local species and made some sketches. After his departure for U.S.A. in 1944, the incomplete work again reverted to the author, who has pleasure in acknowledging the help thus rendered by Mr. Gattani during 1943-44.

ovaries and seeds of *Sicyos*, *Bryonia*, *Momordica*, *Cucumis* and *Lagenaria*. His figures show the division of the ovary and the parietal attachment of the seeds in *Cucumis* and *Cucurbita*.

Referring to *Cucurbita* and *Cucumis*, Mirbel (1815) stated that the ovary is divided into many loculi by radiating placentae the lobes of which are bordered by nerves which bear the ovules, these latter being borne in two ranks on each lobe.

Brongniart (1827) described the anatomy of the ovary of *Cucurbita pepo* with special reference to conducting tissue. He regarded the ovules as being borne on the edges of the carpels and, in general, as representing the teeth of the leaves.

De Candolle is said to have suggested (Lindley, 1853) that in cucurbits the carpellary margins are not curved inward but outward, their midribs being in the axis. He meant thereby that the condition in the Cucurbitaceae is essentially different from what prevails in other plants. Lindley (1853), who cites Wight to have supported such an interpretation, however, refuted such an idea by asserting that the cucurbit ovary was essentially built on the same plan as the ovaries in other plants and that the placentae, which he regarded as parietal, are parts of the carpels and not axial structures as Schleiden (1849) had earlier suggested. He based his interpretation on the horizontal course of vascular bundles between the placentae and the ovary wall.

Referring to a figure of *Cucurbita pepo*, St. Hilaire (1847, p. 877) remarked that the "Cucurbitaceae have neither parietal placentae nor placentae suspended at the top of a single cell, but really three cells and the axile placentae protruding towards the circumference of the fruit".

Eichler (1875) suggested that in *Cucurbita* the ovary is usually composed of three carpels. The edges of these carpels are turned inward, meet at the centre of the ovary and are flexed back towards the outside from the placentae. He represents the placental folds as being separated by a false commissure, evidently formed by the reflexed laminae of the carpellary leaves. In *Ecballium agreste* and *Bryonia dioica* he found a lesser development of the carpellary margins so that the commissural partitions are not so complete.

Kirkwood (1905) quoted Müller as supporting Eichler's conclusion regarding the behaviour of the carpellary margins. He is further said to have cleared up the position of the stigmatic lobes. Each carpel, according to him, is surmounted by a bilobed stigma, but these lobes often fuse in pairs with the adjacent lobes of other carpels and thus the two stigmatic lobes stand on the septae and not on the midribs, that is, they are commissural.

Kirkwood (1905) observed the placentae developing in the region where carpellary margins fuse; but following the interpretation of Schleiden (1849) he regarded them as axial structures. Judson (1929), on the basis of his study of the pistillate flower of cucumber, interpreted the placentae as carpellary, and suggested the same course for carpellary margins as was described earlier by Eichler (1875) and followed later by Rendle (1925).

Thus there is some difference of opinion not only about the nature of placentae — axial or carpellary — but also about the type of placentation in the Cucurbitaceae. While most workers regard it as parietal, others (St. Hilaire, 1847; Willis, 1948; and many text-book writers) describe it as axile. It is my intention to clear up this point as best as possible.

Material and Methods

Most of the material was fixed at Meerut, but some was collected in the U.S.A. and in Europe during 1949-50 when the author was out on a study tour. Dried material, which is indicated in the following table by an asterisk, was heated in a 6 per cent solution of sodium hydroxide for 6-12 hours at 60°C., but this did not always prove to be quite satisfactory. For this reason herbarium material has been used primarily for the sake of comparison. Fresh material gave good contrast with crystal violet and erythrosin, but dried material had to be restained with safranin and fast green to bring out the vascular tissue.

TABLE 1

Tribe[2]	Species	Source
I Fevilleae	1.* *Fevillea cordifolia* L.	Smithsonian Institution (Allard 14478)
	2.* *Alsomitra sarcophylla* Roem. (=*Zanonia sarcophylla* Waltr.)	B.H.[3]
II Melothrieae	3.* *Apodanthera biflora* Cogn.	B.H., E.P.J. 88329
	4.* *Apodanthera* sp.	B.H. 4190
	5.* *Melothria crassifolia* Small	B.H. 4190
	6. *Melothria fulminensis* Gardn.	C. Burch
	7. *Melothria madaraspatana* Cogn.	Meerut
	8.* *Melothria pendula* L.	C.U.H.
	9.* *Melothria scrabra* Naud.	B.H. 675
III Cucurbiteae	10. *Momordica charantia* Linn.	Meerut
	11. *Momordica cochinchinensis* Spreng.	Bot. Garden, Kiel
	12. *Luffa aegyptiaca* Mill.	Meerut
	13. *Bryonia dioica* Jacq.	B. Hor.
	14.* *Ecballium elaterium* Rich.	C.U.H. (Schenk)
	15. *Citrullus vulgaris* Schrad.	Meerut
	16. *Cucumis melo* Linn.	Meerut
	17. *Cucumis melo* var. *ulilissimus* Friield & Gard.	Meerut
	18. *Cucumis sativus* Linn.	Meerut & Ithaca, N.Y.
	19. *Bryonopsis laciniosa* (L.) Naud.	B. Hor.
	20. *Lagenaria vulgaris* Ser.	Meerut
	21.* *Cucurbita palmata* Wats.	C.U.H.
	22.* *Cayaponia agricola* Pueblo	C.U.H. (Barrus)
	23. *Coccinia indica* W. & A.	Meerut
IV Sicyoideae	24.* *Echinocystis fabacea* Naud.	C.U.H. (Dean)
	25. *Echinocystis lobata* Torr. & Gray.	Ithaca, N.Y.
	26.* *Echinocystis macrocarpa* Greene	C.U.H. (Allen)
	27.* *Echinocystis oregana* Kell.	C.U.H. (Cusik)
	28. *Sechium edule* Sw.	C. Burch
	29. *Sicyos angulatus* L.	Ithaca, N.Y.
V Cyclanthereae	30. *Cyclanthera explodens* Naud.	B. Hor.

2. The classification of the family used here is after Müller and Pax (1894).
3. C.U.H. = Cornell University Herbarium, Ithaca, N.Y.; B.H. = Bailey Herbarium, Ithaca, N.Y.; B. Hor. = Bailey Hortorium, Ithaca, N.Y. Name within brackets indicates the author of the sheet.

Observations

The ovary in the Cucurbitaceae consists of 2-5 or more carpels with three as the commonest number. It is doubtful if a really monocarpellary condition, reported by some taxonomists, has yet been obtained in the family although the tendency towards reduction is noticeable, particularly in the tribes Sicyoideae and Cyclanthereae, where pseudomonomerous ovaries occur. The ovary, which is inferior (or nearly so) in all cases, may contain only one seed as in *Sicyos*, or many as in *Citrullus*, distributed in various ways. The form, size and structure of the ovary vary considerably in different stages of development. It is proposed to describe the placental structures of at least one species from every tribe and call attention to other species if and when their structural peculiarities demand it. In all cases, unless otherwise specified, the observations are based on material near about fertilization stage. For obvious reasons attention has mostly been confined to the structure of the ovary in so far as it relates to the placentae.

For the sake of description the structure bearing ovules and contained within

the ovary wall have been designated here as "placental ridges". This expression has been used in a purely topographical sense and does not involve any commitment regarding the morphology of the placenta. For similar reasons the bundles in the inner angles of the placental ridges have been described as "internal bundles" and those occurring outside them in the inner vascular ring as bundles of the "median cylinder".

I — Fevilleae

Fevillea cordifolia — Only two dried flowers (one young bud and one open flower) were available to the author through the kindness of Dr. A. C. Smith[4] of Washington D.C. The following description of vascular anatomy is based on serial microtome sections of the open flower.

In the peduncle of the female flower, there are eight distinct bicollateral bundles forming the vascular cylinder (Figs. 1, 2). As the peduncle swells up, one or more of these may divide and result in ten or eleven bundles (Fig. 3). At about this level there appears a peculiar zone of thick-walled, radially elongated cells resembling somewhat the velamen of an orchid root. It is continuous all round (Fig. 3), occurring 4-5 layers beneath the columnar epidermis and consisting of 5-7 or more cells in thickness. After continuing for a short distance, this thick-walled cylinder breaks up into nine strands alternating with the vascular bundles on the inner side (Fig. 4). Gradually the parenchymatous gaps between these strands widen and through them diverge out the vascular bundles which thus come to be situated in the peripheral region. Soon after, these strands begin to fade away and are finally replaced by homogeneous thin-walled parenchymatous tissue (Figs. 5, 6). The upper part of the flower, which is free from this strengthening tissue, could not be brought back to its pre-pressing condition.

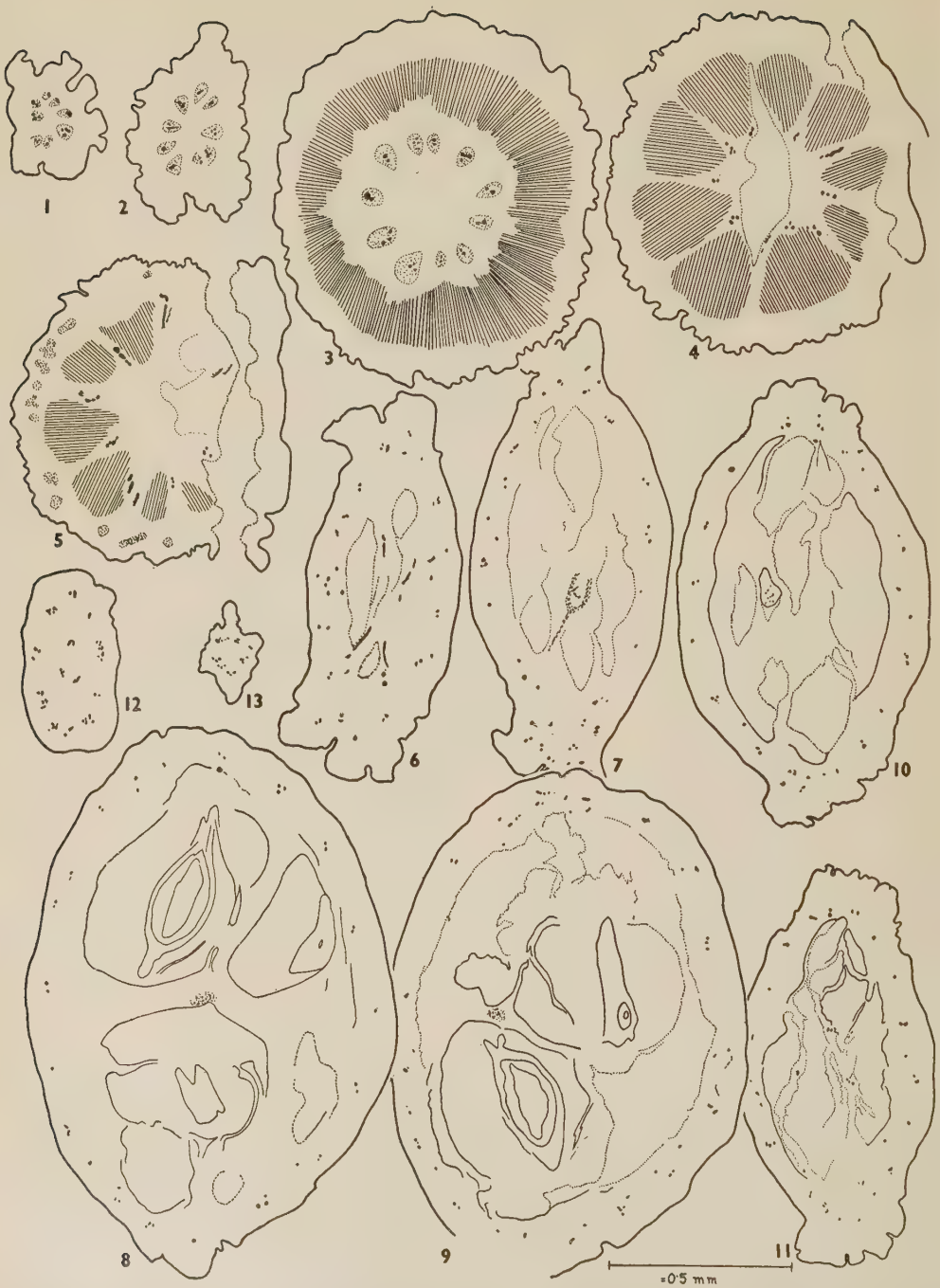
After the passing out of all bundles to the peripheral region, the pith appears to be free from any vascular tissue. But a little distance up, vascular traces from at least four points in the peripheral stele diverge inward almost horizontally (Fig. 6). They fuse in the centre and form a prominent bundle (Fig. 7) whose xylem elements alone are visible. This bundle, unlike the condition in other species, *does not split into separate placental strands* (Figs. 8-10). It is only at the base of the style that it splits into three bundles (Fig. 11). This is a significant feature, and if found to be of general occurrence in the species, it will throw considerable light on the history of placentation in the Cucurbitaceae (see discussion).

After the formation of this central bundle, the three loculi begin to appear, each containing only one ovule (Fig. 8). The origin of ovular traces could not be traced, but there is little doubt that they must have arisen from the central bundle. Towards the end of the ovary, when the central bundle is about to divide, three branches from the peripheral region appear to pass inward and fuse with the central bundle. During the process of this fusion or immediately afterwards, the central bundle (after this fusion it is not morphologically equivalent to the original central bundle) splits into three bundles (Fig. 12). In the stylar region they are joined on alternate radii by three bundles from the peripheral ring (Fig. 13). In all probability, these last arrivals are the dorsal bundles of the three carpels. All these six bundles show some anastomosing and branching before finally disappearing in the stigma.

II — Melothrieae

Apodanthera sp.— Only one female flower of this species was available. Figs. 14-22 are based on serial transverse sections of this specimen. The peduncle, which passes into ovary rather gradually, contains a ring of about 16 bicollateral vascular bundles (Fig. 14). These bundles show some anastomosing and branching, and then some fine branches from them appear to diverge into the parenchymatous pith just below the ovary region (Figs. 15-16). Most of these finer ramifications fuse into a central plexus of vascular tissue (Fig. 17), in which the

4. Some other friends from New Zealand and South America provided herbarium material of this species, but all of it turned out to be male!



FIGS. 1-13 — Serial transverse sections of pistil of *Fevillea cordifolia* from base upward. Note the single central bundle (in place of three internal bundles) in Figs. 8-10. For explanation, see text.

xylem is distinguishable mostly towards the periphery. A little higher up, this central plexus splits into five internal bundles around which differentiate the five large and massive parietal placentae (Figs. 18, 19). These internal bundles, particularly in the upper region, are inversely oriented having their xylem towards the periphery (Figs. 20, 21).

Beyond the ovule-bearing region, the internal bundles diverge out slightly and appear to fuse with some bundles from the median cylinder (Fig. 22). They continue into the style along with the dorsals.

Melothria fulminensis — In this species the peduncle which is very narrow swells up suddenly into the ovary. It contains only five vascular bundles within a cylinder of sclerenchyma which disappears after traversing for a short distance. In the base of the ovary the vascular bundles begin to diverge out almost horizontally dividing as they do so. Some of the branches lag behind and fuse into a central plexus of vascular tissue. Not long after its formation, this central plexus, unlike the condition in *Fevillea cordifolia*, splits into three internal bundles which appear to be inversely oriented. The ovules are so arranged that in one section, generally, only one appears to be most prominent. Such a condition pushes the internal bundles to one side and makes them appear somewhat excentric. In young fruits the ovary is all solid and each ovule is enclosed in its own cavity. It is thus difficult to make out the boundaries of the three placentae.

III — Cucurbiteae

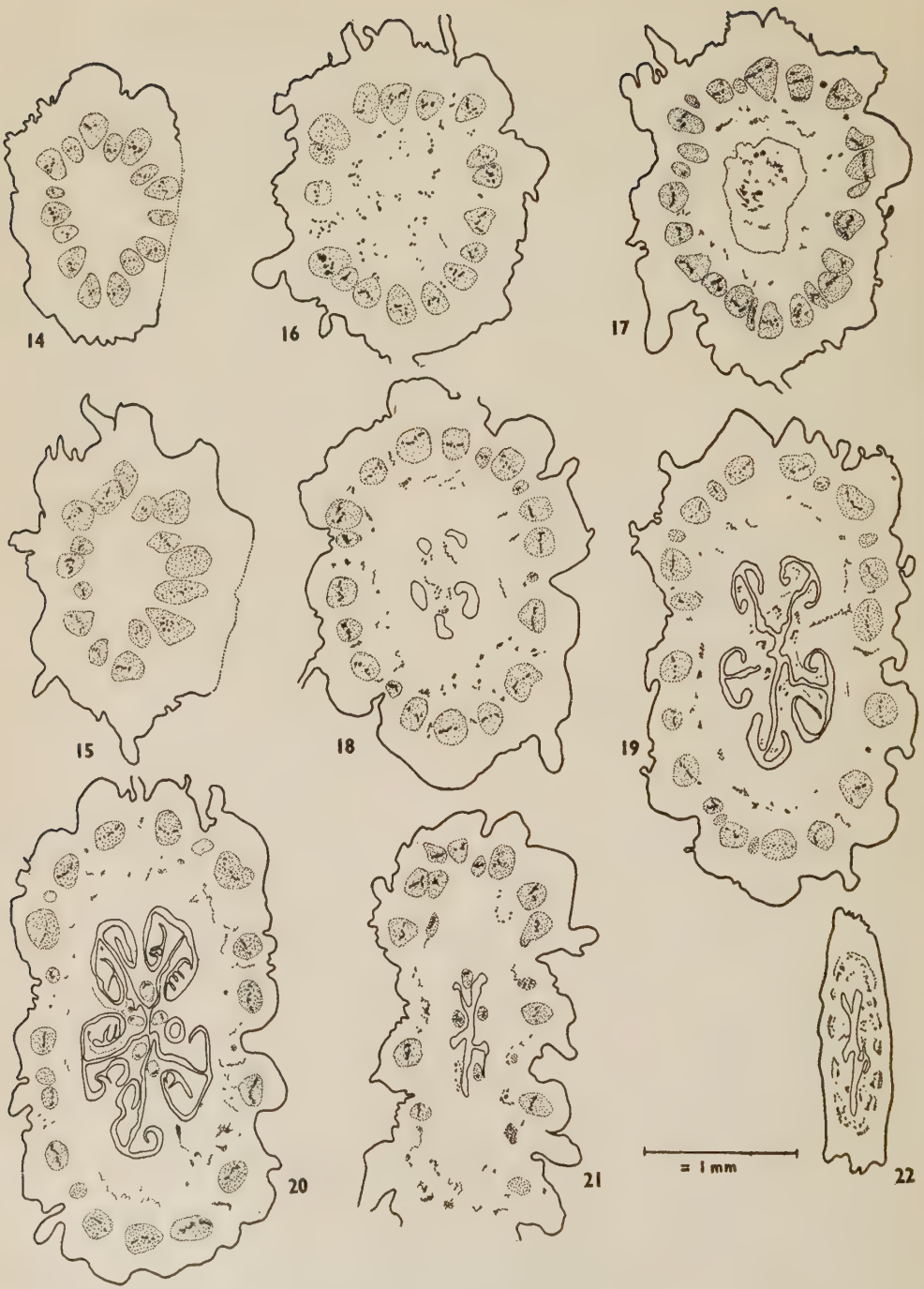
Bryonopsis laciniosa — The tricarpellary ovary in this species is more or less globose and develops into a spherical or ovoid-conical green berry about ¾ in. across with white longitudinal stripes all round. It contains about six ovules arranged in such a way that in one section only three belonging to alternate carpellary margins can be seen. The following observations are based on young fruits.

A cross-section of the peduncle, just beneath the ovary of the female flower, shows five bundles arranged in a narrow ring in the centre. Higher up, where the peduncle swells up into the ovary, these bundles diverge out and come to occur near the periphery. Some of the finer branches form a triradiate central plexus which shows broken connections with some of the outer bundles. When the ovules begin to appear, these connections are lost and the central plexus splits into three internal bundles as in the last species. During their upward course, these internal bundles become inversely oriented and give off several ovular traces which pass out toward the ovule-bearing region. Only one from a margin probably enters the ovule and the rest are used up in the parenchymatous tissue of the placental region.

Above the ovule-bearing region every one of the internal bundles again gets connected with certain branches in the median cylinder with which they ultimately fuse. The three fusion products alternate with the three carpellary dorsals which have in the meantime diverged in from the periphery. Thus the style receives six bundles, three dorsals and three ventrals.

Cucumis sativus — Vascular anatomy of the cucumber female flower has already been described by Judson (1929). But as some of his findings, and particularly his conclusions, are at variance with those of the present author, it may be worth while to re-state the more important anatomical features of this species.

In the peduncle of the female flower there are ten bicollateral vascular bundles arranged in a ring (Fig. 23). As the peduncle starts swelling into the ovary, the main bundles diverge out. In doing so, they divide anastomose and redivide and contribute numerous small branches which lie scattered in the centre (Figs. 24, 25). Out of this medullary mass one bundle in the centre becomes more prominent than the rest (Figs. 26, 27). This is concentric to begin with. At this level most of the vascular tissue resolves itself into two more or less distinct and concentric zones or rings (Fig. 27). The outer zone contains the ten large bundles and numerous other small ones. This contributes the carpellary dorsals and traces for the perianth whorls. The



FIGS. 14-22 — Serial transverse sections of pistil of *Apodanthera* sp. from base upward.

median zone, which is only meant for the carpels, consists of numerous small bundles (provascular bundles?) which are in a state of constant division and anastomosis. As a result of this, there is no single bundle that can be made out here for any appreciable distance. At three more or less equidistant points, the ramifications in this median ring are connected to the central concentric bundle or plexus through some horizontal 'broken' branches (Fig. 27).

Higher up, when the three placentae appear, the central plexus splits into three bundles that are separated apart from one another by a triradiate slit in transverse section (Fig. 28). These slits extend outward and finally join the tangential cavities outside, thus delimiting completely the three placentae from one another (Figs. 29, 30).

Vascular supply for the ovules is interesting. Frequently a branch from the "median cylinder" diverges in almost horizontally. On approaching the "internal" bundles, which have in the meantime become distinctly inversely oriented, it splits into two halves which swing outward and backward in opposite directions (Fig. 29). While diverging out along the edge of the placental ridge, they may receive branches from the internal bundles. The "compound" traces so formed enter into the ovule-bearing region. Sometimes branches from the median cylinder and internal bundles enter this region separately. But as these traces branch repeatedly before reaching the ovules, it cannot be definitely asserted as to which of them enter the ovules and which disappear in the tissue of the placenta. One who has seen cucumber sponges from dried fruits can appreciate such difficulties much better. However, it seems probable that ovules in this species generally receive "compound" vascular supply (contributed by both the median and internal bundles) and only occasionally "simple" supply (contributed either by the "external" or internal bundles (cf. Fig. 98).,

Another interesting feature which becomes clear after an inspection of Figs. 29-32 is that the region which bears the ovules is more or less distinctly marked off from the septal tissue in which the ovules are buried.

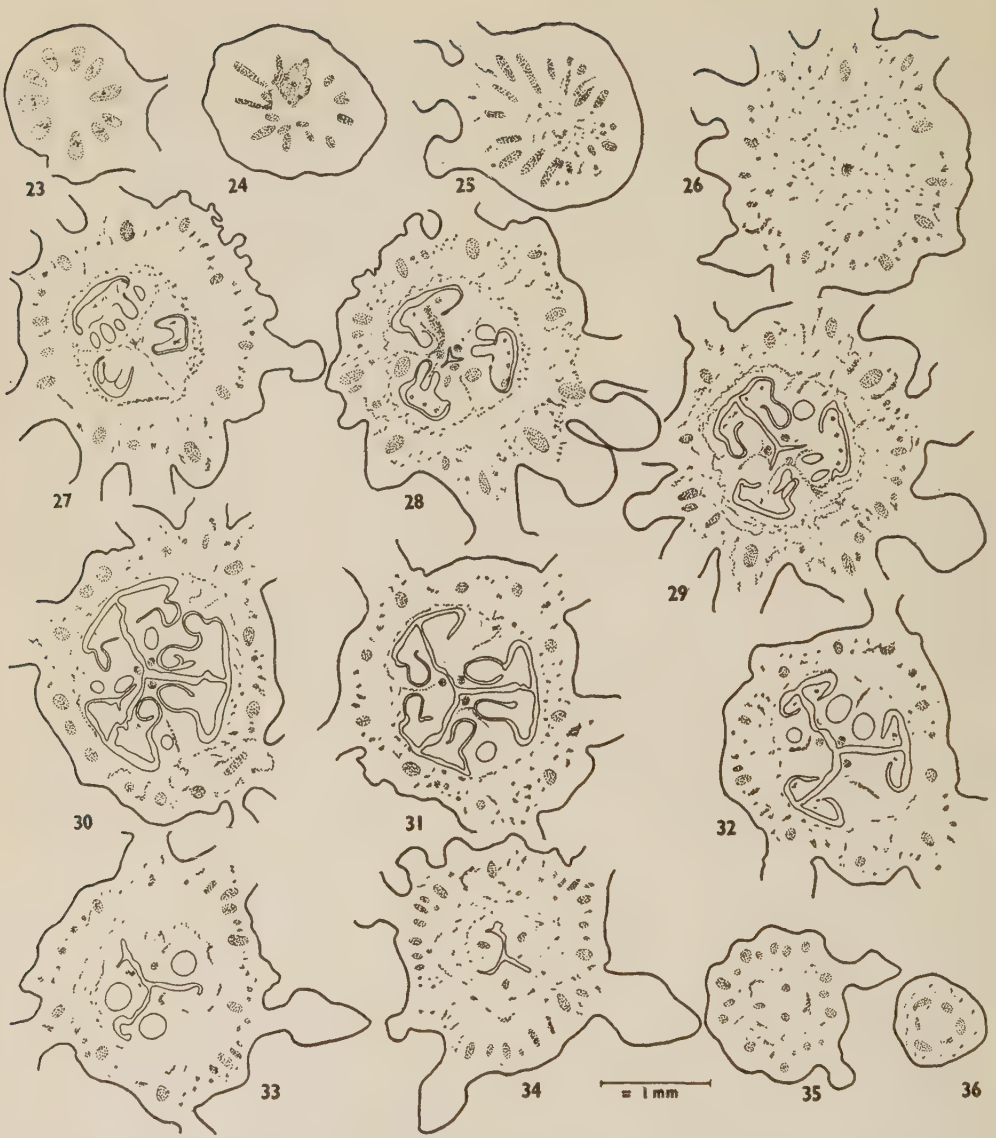
Higher up, beyond the ovule-bearing region, the bundles of the median cylinder begin to fade away (Figs. 33-35). The internal bundles continue into the style where they are joined on alternate radii by the three dorsals (Fig. 36).

Citrullus vulgaris — The peduncle of the female[5] flower swells up suddenly into the ovary. A transverse section through the base of the ovary shows three equidistant, tangentially elongate slit-like cavities. As they widen upward, there descend into them, for a short distance, the free basal ends of the three placental ridges (Fig. 41). A little upward, these slit-like cavities extend laterally towards one another and the placental ridges get attached on the inner side (Fig. 42). Soon after, these cavities become T-shaped and finally merge with one another in the centre (Figs. 43, 44). The central region of the ovary is thus divided into three vertical columns — the placental ridges — which are arrow-head-like in transverse section. The ovules are borne on three placental ridges on the sides just adjacent to the circumference of the ovary wall (Fig. 44). They occur in 4-6 or more vertical rows on either side of a placental ridge and are more or less buried in its tissue. It will be noted here that the ovule-bearing edge has a tendency to curve out away from the septal side of the placental ridge.

Above the ovule-bearing region the ovarian cavity around the "placental ridges" gradually becomes obliterated, the tangential parts of the T-shaped cavities disappearing earlier than the radial parts (Fig. 46). The resulting solid style then splits up into three stigmas, each becoming bifid at the apex (Fig. 51).

There are about ten bicollateral vascular bundles in the peduncle of the female flower (Fig. 37). They branch, anastomose and rebranch and thus form many bundles scattered irregularly (Fig. 38). While the major branches pass outward, the smaller ones migrate

5. The flower whose sections are figured here had two functional stamens.



FIGS. 23-36 — Serial transverse sections of a young fruit of *Cucumis sativus* from base upward.

in (Figs. 39, 40). Some of these latter become scattered in the medullary region of the receptacle and then arrange themselves in the form of a triradiate plate in transverse section (Fig. 41). Just in the centre of the ovary one bundle becomes more prominent than the others (Fig. 42). Soon after its formation it splits into six more or less distinct bundles which arrange themselves in the form of a cylinder and which appear to be normally oriented (Fig. 43). A little higher up, this medullary cylinder disappears leaving only three internal bundles with inverse orientation (Fig. 44). Each of these latter gives out one or more branches on either side which, if well differentiated, also show inversion. Besides, there is one additional bundle in each of the outer angles of the placental ridges. They are,

however, transitory and may be replaced from time to time by new ones from the internal bundles (Fig. 44).

Bundles occurring outside the placental region are too many and scattered. They are arranged in two more or less distinct regions, the outer region consisting mostly of prominent bundles and supplying traces to peripheral organs and the median cylinder consisting of small branches just outside the placental ridges (Figs. 42-44). This latter constitutes the vascular supply of the ovary. Beyond the ovule-bearing region some of its branches approach towards and fuse with the internal bundles (Fig. 45). At this level the median cylinder may be reinforced by certain branches from the peripheral ring (Figs. 46, 47).

The ovular traces appear to be directly connected to the branches in the median cylinder. At frequent intervals paired traces from the median cylinder diverge almost horizontally inward and, after traversing for some distance, swing outward and backward in opposite directions following the contour of the placental ridges till they reach the ovule-bearing region (Figs. 44, 97). In so doing they generally do not contact the internal bundles although their lateral branches may sometimes be " passed through". Once in a while, branches from the median cylinder may directly contact the internal bundles which in turn may give out branches on either side. These latter meet the traces which are swinging out directly from the median cylinder into the ovule-bearing region (Fig. 44).

The ovular supply is, therefore, largely obtained directly from the median cylinder and the internal bundles appear to play rather an insignificant part in this connection. In fact, some lowest ovular traces are given out when internal bundles have not yet differentiated (Fig. 42). This appears to be a very significant point in determining the type of placentation, and attention will be drawn to it while discussing that topic later on.

Beyond the ovule-bearing region of the ovary, the three internal bundles are separated from one another by a triradiate opening (Fig. 46). Their lateral branches fade away (Fig. 47). The median cylinder now differentiates into a number of prominent bundles which form a distinct ring outside the internal bundles (Fig. 48). In the stylar region this ring dissociates into three more or less distinct arches of vascular tissue with which the three internal bundles fuse (Fig. 49). The three stigmas are carinal and each receives a number of vascular bundles (Figs. 50, 51) which soon disappear.

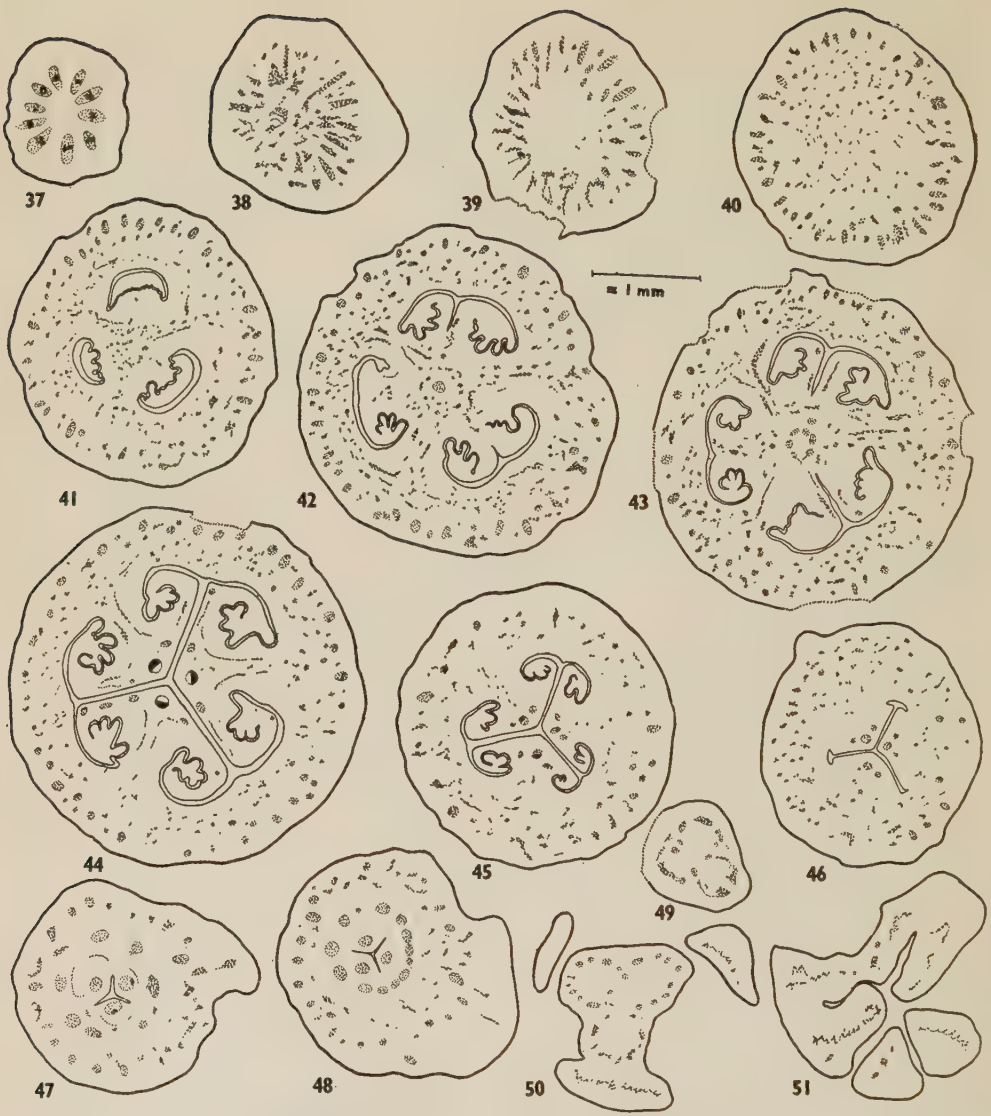
Coccinia indica W. & A. (=*Cephalandra indica* Nad.) — Female flowers are few as compared to the male and have a peduncle ¼ in. long which is about one-fourth of the male peduncle. The young ovary is oblong and more or less irregular in outline. As it develops into a fruit, it becomes smooth. The style is comparatively long and ends in three bifid stigmas. There are three vertical placentae which are free throughout their length. The mature fruit is a cylindrical oblong, bright red berry which has numerous seeds embedded in the red pulp.

In its upper region the peduncle is flattened and distinctly bilobed. It contains about ten prominent bundles occurring alternately in two concentric rings as in the vegetative stem. The outer bundles diverge out a little while the inner ones appear to migrate inward and, after dividing and redividing, form the median cylinder and the central plexus. The central plexus, as usual, splits into three internal bundles each occupying the inner angle of one of the placental ridges. Occasionally they may be connected to the bundles in the median cylinder through some more or less horizontal branches.

A little higher up the slits separating the placental ridges get obliterated and the three internal bundles are joined on alternate radii by three prominent bundles from the outside. Thus in the style there are six bundles which divide and redivide and form a continuous cylinder of vascular tissue that later on splits up into three arches, one for each stigma.

IV — Sicyoideae

Echinocystis lobata Torr & Gray [=*Micrampelis lobata* (Michex) Greene]. The plant is monoecious but the female flowers occur singly and are very rare. The ovary is ovoid and covered with



FIGS. 37-51 — Serial transverse sections of a young fruit of *Citrullus vulgaris* from base upward.

many more or less prominent spinous outgrowths projecting upward. The style is short and stigmas are lobed. The ovary is unilocular in young condition but soon after fertilization the placentae meet and fuse in the middle, particularly in the lower region where the ovary often appears bilocular.

In the peduncle of *Echinocystis lobata* there are only five bicollateral vascular bundles to begin with (Fig. 52). However, when the peduncle swells up into ovary, they divide into about ten bundles (Figs. 53, 54), which in turn break up into numerous small branches scattered all over in the peripheral region (Fig. 55). From two opposite ends some of the finer branches diverge in towards the pith (Figs. 56, 57). On the remaining two sides there appear two semicircular (in

t.s.) loculi which are occupied by the free hanging parts of two richly vascularized placentae. In Fig. 56, one of the two placentae is already attached to the central column. It must be noted that at this level the two placentae are oriented in a plane at right angles to that of the placentae in the upper region (cf. Fig. 62). This is indeed a significant point and we shall return to it in discussion.

Each half of a placenta now bears a single ascending ovule which is enclosed separately by accrescent and locellate placenta. Thus in transverse section a young fruit shows four chambers each having one ovule (Figs. 57, 58). All around the ovule the tissues are richly vascularized. By this level two bundles in the central region become more prominent. Each of them forms a part of the vascular system of its own side (Figs. 58, 59), and ultimately fuses with the other in the centre (Fig. 60). This fusion bundle continues only for a short distance, and then it splits again into two bundles which occupy positions on opposite sides (Fig. 61). These two bundles, which correspond to internal bundles of the other species, are definitely inverted, and the two placentae separate apart in between them (Fig. 62). In the upper region of the ovary the vascular network about the ovule-bearing region appears to contract and finally disappear (Figs. 63-65). The internal bundles continue into the style where they are joined by the carpellary dorsals (cf. Fig. 66).

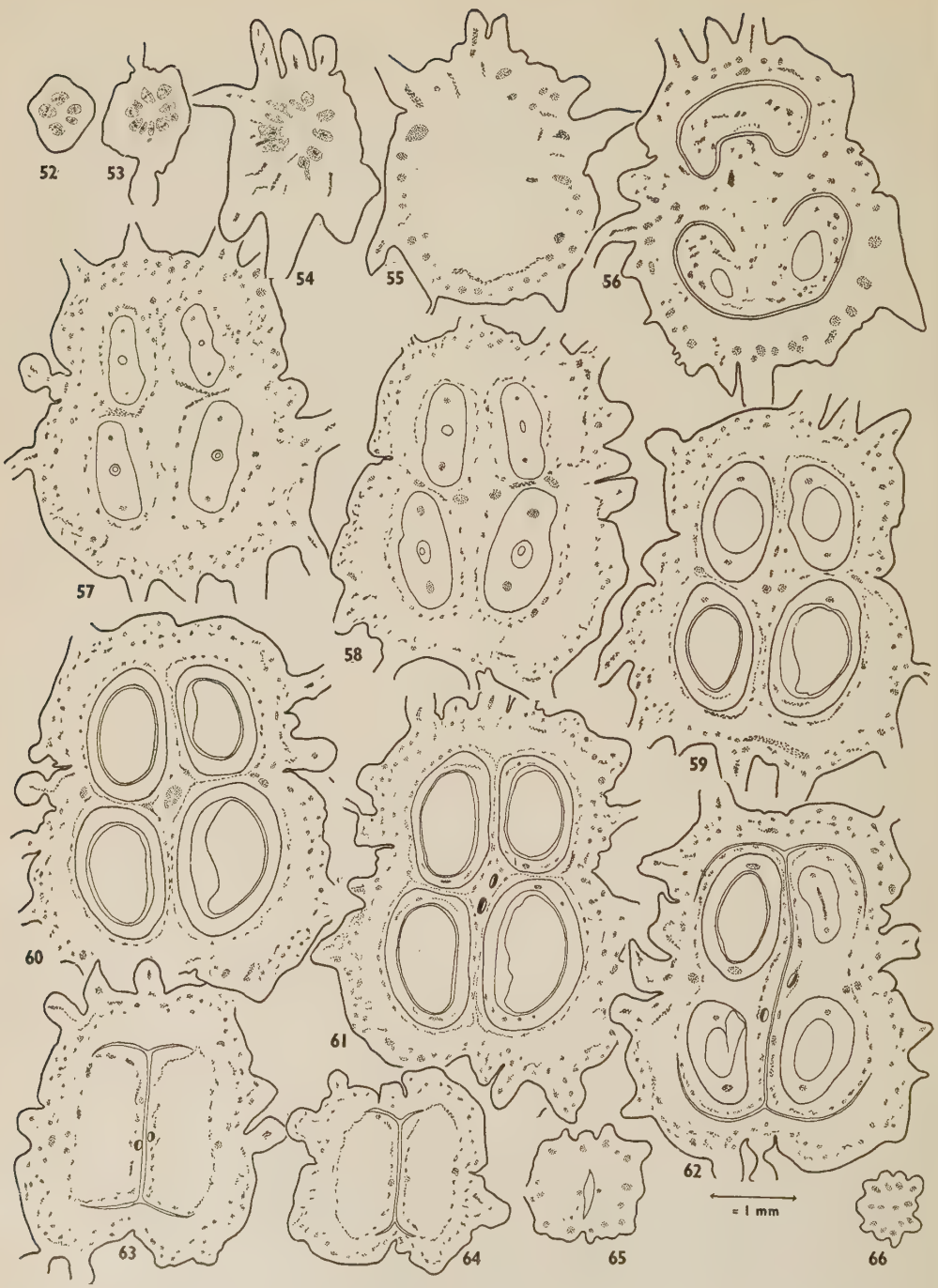
In all the species of *Echinocystis* examined there are prominent massive protuberances arising from the surface of the ovary. Surprisingly enough, all these processes, like similar ones in *Cucumis sativus* and *Sicyos*, receive some vascular supply. For this reason they cannot be regarded as just hairy enations from the wall of the ovary.

In *E. macrocarpa* while one of the two flowers examined was perfectly similar to the one described above, the other had apparently a single carpel each margin of which bore two ovules one above the other.

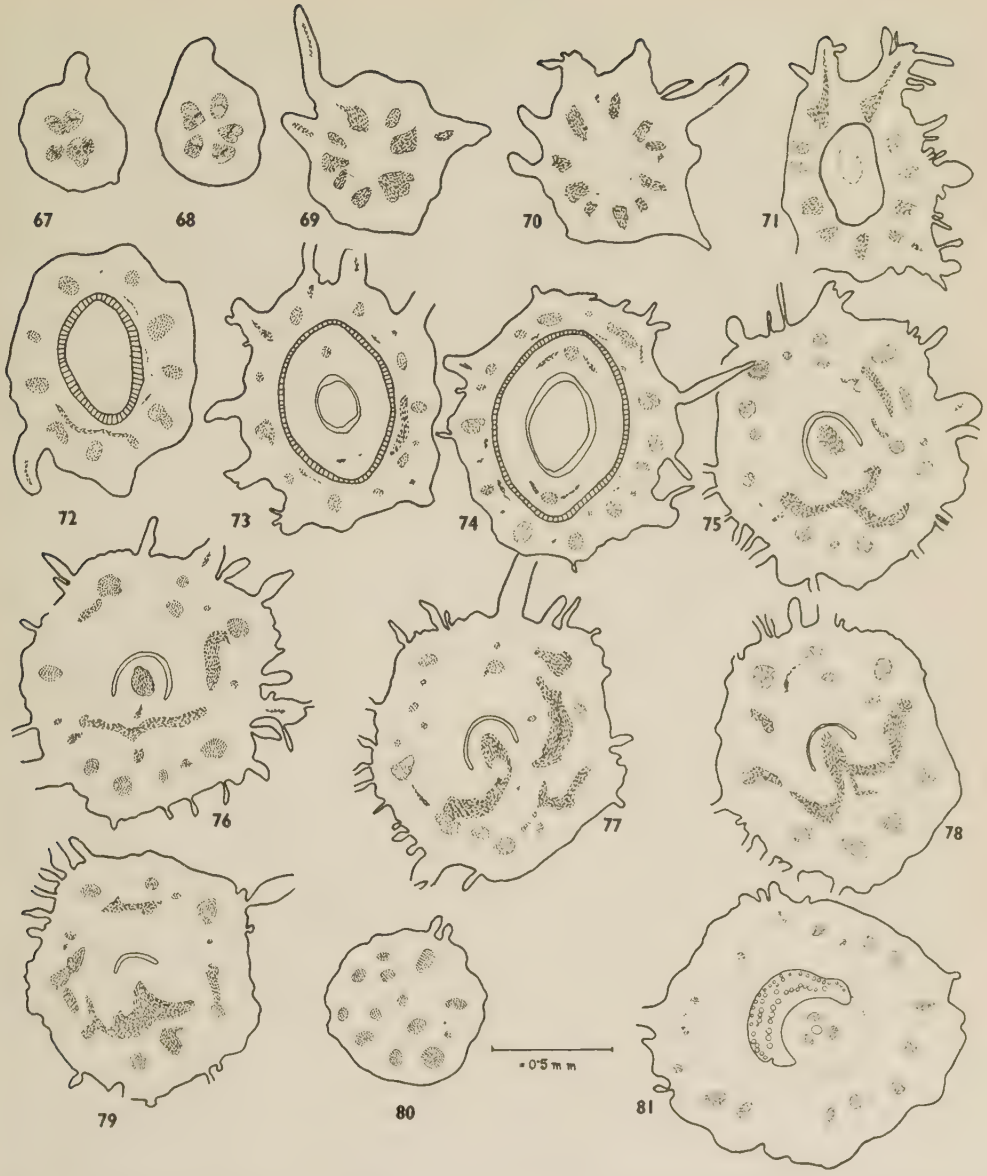
Sicyos angulatus — The ovary in *Sicyos angulatus* is oblong or fusiform and passes into a slender, erect style which dialates at the apex into three stigmatic lobes. It is unilocular and bears a single suspended ovule filling the whole space. The ovule hangs vertically down and is provided with two integuments, the inner of which is only two layered and very insignificant in young seeds. The outer integument is, however, very prominent with the inner epidermis and hypodermis becoming highly columnar, particularly in micropylar region.

The peduncle in a young fruit contains five vascular bundles (Figs. 67, 68). Higher up where it passes into the ovary and where the lowest chalazal part of the ovule makes its appearance, the ovule being pendant, these bundles divide into ten, the alternating ones being generally larger than the others (Figs. 69-72). In the chalazal end of the ovule one vascular bundle becomes visible as serial transverse sections are viewed from base upward. This stretches itself outward on either side and very soon two arc-shaped bundles are produced at opposite ends (Figs. 73, 74). These are actually parts of the same bundle which enters the funiculus and raphe of the ovule from the top of the ovary and, on reaching the chalazal end, crosses over to the other side and assumes an upward course in the outer integument and continues right up to the micropyle. The single ovule trace appears to be contributed from three different sources located at considerable distance from one another (Figs. 75-79). Below the stylar region four bundles are noticeable surrounding the conducting or transmitting tissue. Two of these which are larger and occur opposite to one another appear to be concentric. A little higher up, the two smaller bundles disappear and instead a third bundle appears. These three bundles now become equidistant and enter the style (Figs. 80, 81). In all probability they are the carpellary dorsals. This is partially borne out by the fact that they are separated from the outer bundles above the region of the ovary, as in other genera. If this is so, it is obvious that there are three carpels in *Sicyos*, a fact also borne out by the presence of three stigmas.

The ovary and fruit in this species also are covered over by prominent



FIGS. 52-66 — Serial transverse sections of a young fruit of *Echinocystis lobata* from base upward. Note the plane of orientation of placentae in the lower and upper regions (cf. Figs. 56 and 62).



FIGS. 67-81 — Serial transverse sections of a young fruit of *Sicyos angulatus* from base upward. Note the vascular supply of the single pendant ovule being obtained from three sides (cf. Fig. 78).

vascularized protuberances, whose morphological nature is not easy to determine.

V — CYCLANTHEREAE

Cyclanthera explodens — A young fruit of *Cyclanthera explodens* is obliquely ovoid. It is sparingly covered by thick, spinous, erect hairs. The neck is very short and narrow and the stigma is terminal and discoid. In the interior there is a single massive placenta borne slightly to one side bearing 4-6 ovules, each enclosed in a separate cellule.

The peduncle of the female flower, which is somewhat flattened, contains a ring with about ten prominent vascular bundles (Fig. 82). They divide and some of the finer branches diverge into the pith (Fig. 83). Higher up, more branching takes place and a dense cylinder of vascular tissue is produced (Fig. 84). At this level the loculus appears containing the free hanging part of the placenta and an ovule. It must be noted here that the placenta is crescent-shaped and excentric, and that the small medullary bundles are connected to the peripheral ring only on the side opposite the one on which the placenta is located. Each placenta carries six ovules which are contained separately in their own cellules (cf. Figs. 85-88). From each ovule which is pendant one bundle appears to enter the placenta (Fig. 86). A little higher up all the separate ovular traces fuse into a plexus, which in fact is the real placental tissue from which ovular traces descend (Figs. 87, 88).

Beyond the ovule-bearing region this plexus gets connected with the bundles on the other side across the centre (Figs. 89-92). A little below the style it appears to divide into two bundles (Figs. 93, 94). They are apparently the internal bundles which are soon joined by some branches from the periphery. All these divide and form a faint cylinder of vascular tissue in the style (Fig. 95).

Discussion

Vasculature of the Placental Ridge and the Ovular Supply — In all the species studied here, there is one, more or less prominent, vascular bundle in the inner angle of each placental ridge. This is invariably inversely oriented with reference to the central axis of the flower, and in the preceding account is referred to as ' internal bundle '. In its upward course it gives off a varying number of branches right and left passing outward into the ovule-bearing regions. The number of these branches is roughly proportional to the number of ovules in different species.

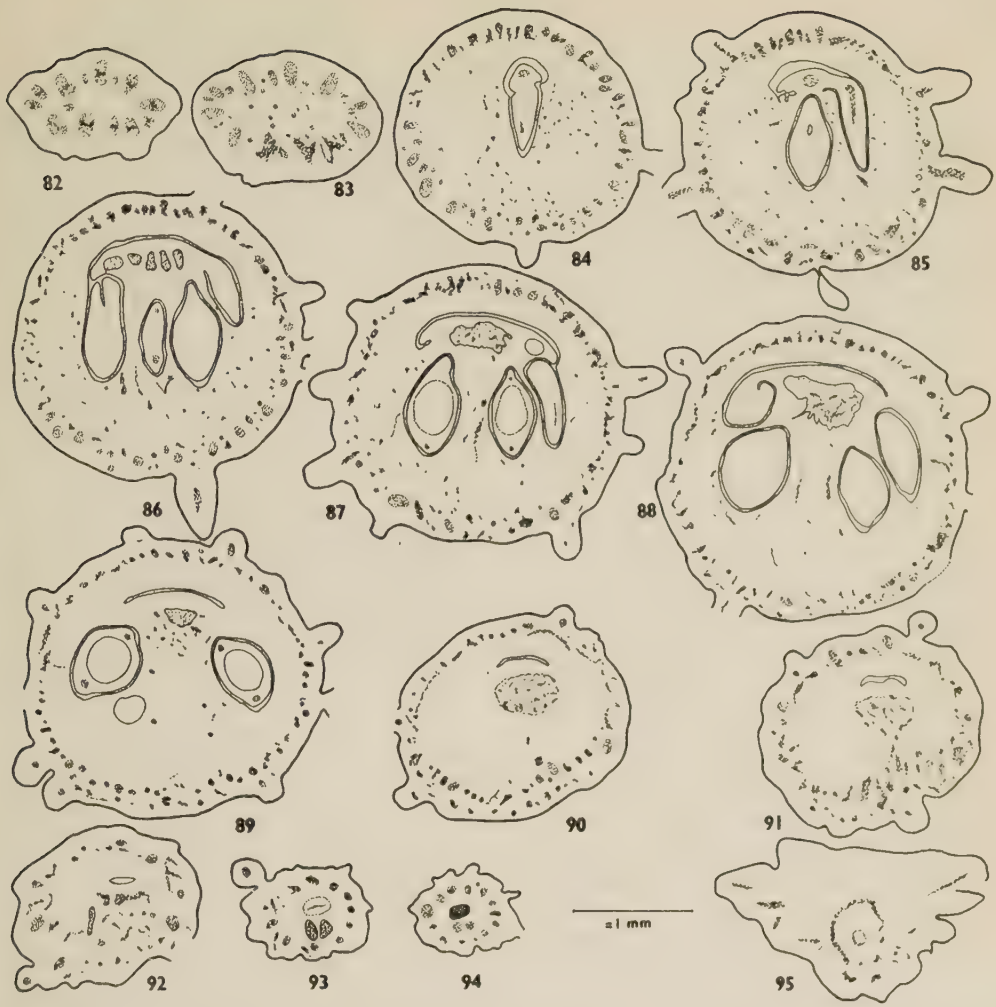
In the inner region of the ovary wall there is the median cylinder of very small vascular bundles which vary in number considerably. Both below and above the fertile region of the ovary this cylinder is often connected with the ' internal bundles ' through those of its branches which occur on their radii. Beyond the ovule-bearing region, as a rule, most of these bundles disappear; the remaining ones fuse with the internal bundles which have by now approached them. The three fusion products become normally oriented during their upward course, and in the stylar region alternate with the three dorsals which have in the meantime differentiated from the peripheral ring. Further upward these six vascular bundles divide and redivide, forming three more or less distinct arcs (in t.s.) of vascular tissue.

From the description of the various species it is apparent that there is some variation in the origin of ovular supply. For the sake of description we can distinguish three possible ways in which traces for the ovule-bearing region are given out. In *Bryonopsis* everyone of the three internal bundle sets off traces right and left. One of these enters the ovule borne on each side of a placental ridge (there being only six ovules in all, one on each side of a placental ridge) while the others disappear in the tissue of the placental column (Fig. 96).

In *Citrullus vulgaris,* where there are numerous ovules, majority of the ovular traces appears to arise in pairs from the median ring of vascular bundles. They diverge in more or less horizontally and then before reaching the internal bundles swing outward and backward to the ovule-bearing region (Fig. 97). Occasionally, however, the internal bundles also give off a few branches to the ovular zone.

The third condition is apparently intermediate between the preceding two. Here the branches from the median cylinder appear to come very near the internal bundles and when, on splitting radially, their branches swing right and left, outward and backward, they may frequently be joined by small branches from the internal bundles (Fig. 98). Such a condition is most common in *Cucumis sativus, Cucumis melo,* etc.

The ovular supply in bi- and penta-



FIGS. 82-95 — Serial transverse sections of a young fruit of *Cyclanthera explodens* from base upward.

carpellary gynaecea is somewhat different. In *Apodanthera* sp., for instance, all the ovular traces are derived from the internal bundles and the same is the condition in *Fevillea* and *Cyclanthera*. In *Echinocystis lobata*, on the other hand, traces for the sub-basal ovules arise probably from the median cylinder at a level where the internal bundles have not yet differentiated.

Thus there is a lot of variation with respect to ovular supply and this seems to indicate that the placentation in the Cucurbitaceae is still in a ' fluid ' state and that nothing is finally ' fixed ' as yet.

INTERPRETATION OF THE INTERNAL BUNDLES — A correct interpretation of the internal bundles is of vital importance to the understanding of the mode of placentation in the Cucurbitaceae. There are two ways in which they may be interpreted: (1) they are just branches from the median cylinder in which are incorporated, as it were, the ventral bundles as well; or (2) they are themselves the ventral strands.

Before taking up a detailed consideration of these alternatives, it will be worth

FIGS. 96-98 — Diagrammatic representation of vascular supply (dotted lines) to the ovule-bearing regions. The three inverted bundles in the centre represent the internal bundles and the small bundles in the inner region of the " wall " of the ovary are the bundles of the median cylinder. Cavities are represented by thick black lines.

while to define the various bundles of a compound gynaeceum. The ventral strands are formed by the fusion of ventral bundles of carpellary margins. As a rule they alone furnish ovular traces and have thus often been called placental strands. In ' closed ' carpels (and also in certain ' open ' carpels; see Puri, 1951, 1952a) they are generally inverted with reference to the floral axis and occur on the same radii as the midrib bundles. The dorsal bundles are the midrib bundles which usually continue into the style and stigma and, as a rule, have no connection with the ovular supply. In between the dorsal and the ventral bundles there may be one or more median laterals. Corresponding median laterals of adjacent carpels often fuse into median marginal strands or septal strands. Like the dorsal bundles they also contribute little towards the ovular supply and are normally oriented.

Bearing these features in mind let us examine the first alternative given above. For the sake of argument let us assume that the ventral bundles are incorporated, as it were, in the median ring, and that the internal bundles are just branches therefrom. Such an assumption seems to be called for by the fact that the median cylinder of vascular tissue often contributes traces to the ovular region — a function of the ventral bundles. Now since the ventral bundles are commonly held to mark the approximate limits of carpellary margins, it follows that the carpellary margins remain on the periphery, and that the placental ridges are just secondary outgrowths arising from them. Many taxonomists, including Payer and Baillon (1872), Le Maout and Decaisne (1876), Trimen (1894), Prantl (1897), Bonnier and Sablon (1919), Haines (1922), Warming and Potter (1932), speak of the cucurbit gynaeceum as spuriously trilocular. If they are using the word ' spurious ' — an unfortunate expression which should be replaced by 'secondary' — in the ordinary sense[6], it is possible that they imply faith in the interpretation outlined above. With reference to Cucurbitaceae and Ericaceae, Eames & MacDaniels (1947) have distinguished between the ventral strands and the placental strands. According to them, in large and massive placentae these two expressions are not synonymous.

One serious difficulty in accepting this interpretation of the internal bundles is their inverse orientation. The present author has considered this as an important structural feature although he is well aware of the growing tendency among morphologists to ignore its importance (see Eggers, 1935; Arber, 1938). Besides, the internal bundles are usually more

6. Hooker (1897) seems to be using it in a different sense when he writes: " The edges of the carpellary leaves being often turned in so far that the ovary (in flower time even) is spuriously three-celled."

prominent than any of the bundles in the median cylinder and they never appear to be given off as a single bundle from the outer ones. Rather, as in *Citrullus vulgaris*, they sometimes arise from a distinct central ring. Thus, if their prominence, mode of origin and orientation are of any consequence, the internal bundles probably deserve a more independent status than is given to them in this interpretation.

In view of such considerations the second alternative, in which the internal bundles are treated as ventrals, appears to be more convincing. Here, however, the bundles of the median cylinder seem to present some difficulty. As has already been pointed out, some of the branches, which occur on the same radii as the internal bundles, also furnish ovular traces, a function which, as a rule, is performed by ventral strands and is rather unusual for other bundles. It seems best to describe them as branches of the secondary marginal (septal) strands. A close parallel appears to exist in the related family Caricaceae. In *Carica papaya*, where the ovules are numerous and scattered over most of the inner surface of the ovary wall, the ovular supply is found to be derived from more than one source (Devi, 1952). In the lower region of the ovary, bundles interpreted as ventral (placental) strands furnish ovular traces. Above, about the middle of the ovary, however, the bulk of the ovular supply is derived from other bundles interpreted as median marginal strands. Some cases are also on record where ovular traces are contributed even by the dorsal bundles (see Saunders, 1936; Bailey & Nast, 1945), but this is not quite relevant to our point here. What needs emphasizing is that in some cases bundles other than the ventrals may also furnish ovular traces (see Puri, 1952b). In *Citrullus vulgaris*, where there are so many ovules, this calling into service, so to say, of the median laterals for furnishing ovular traces is easily understandable on purely physiological grounds.

In the present state of our knowledge, therefore, it seems appropriate to interpret the internal bundles as ventral strands (cf. Judson, 1929).

Nature of the Placental Ridges — There are three possible interpretations of the morphological nature of the placental ridges: (1) they are inward outgrowths from the carpellary margins which themselves remain at the periphery (Fig. 99); (2) they are outgrowths arising from the in-turned carpellary margins (Fig. 100); and (3) they are carpellary structures formed by the curving in and swelling of the carpel margins (Fig 101).

A discussion of these views involves a consideration of the fundamental nature of placenta. We have to determine, for instance, whether the ovules are always borne on carpellary margins or whether in some cases they can also develop on some outgrowths arising from them. There is no easy way of settling this issue for the simple reason that it cannot be

Figs. 99-101 — Diagrammatic representation of the three possible interpretations of the nature of the placental ridges. The author's view is illustrated in the last figure. Dotted region represents carpellary tissue and hatched portions are outgrowths from carpel margins.

determined where exactly the carpellary margins end and the placental outgrowths, if any, begin. No doubt, ventral strands do help us in delimiting the boundaries of the carpellary margins but such help is obviously of limited value.

In some cases, as in certain ranunculi, the placenta is just a position on the inner surface of the ovary wall to which the ovule is attached (Eames & MacDaniels, 1947). In others, as in certain species of the Capparidaceae, Violaceae, etc., it may be just a more or less prominent swelling of the carpellary margins. This is indicated by the fact that in all such plants the carpellary ventrals or their fusion products, the ventral strands, occur almost in the middle of the placental column.

In still other cases it is claimed that the placentae are more or less distinct structures, borne on carpellary margins *as outgrowths and not as mere swellings of the margins*. This is believed to be indicated firstly by the prominence of the placentae and secondly by the fact that the carpellary ventrals do not enter into them which would have been the case if the placentae were mere swollen margins. The fact that these ventrals remain on the periphery has been interpreted (Wilkinson, 1944; Heinig, 1951) to indicate that carpellary margins also end there, and the structures which proceed inward are simply outgrowths from them. On fusing in the centre, these ovule-bearing outgrowths may falsely or spuriously divide the ovary into loculi. Such a condition has been described in certain species of the Cornaceae (Wilkinson, 1944), Thymelaeaceae (Heinig, 1951), etc.

Acceptance of such an interpretation will materially modify our usual concept of the carpel. So far it is generally believed that ovules are borne on carpellary margins, but in view of the suggestions of Wilkinson and Heinig we will have to admit that in certain cases they may as well develop on outgrowths arising from the carpellary margins. In other words, we have to conceive of placentae as structures different from carpellary margins.

Let us now consider separately the three possibilities outlined above. Regarding the first suggestion, it is possible that some taxonomists who speak of the cucurbit gynaeceum as spuriously trilocular (e.g. Payer & Baillon, 1872; Le Maout & Decaisne, 1876; Trimen, 1894; Prantl, 1897; Bonnier & Sablon, 1919; Haines, 1922; Warming & Potter, 1932) believe that a condition similar to the one described above for the Cornaceae and Thymelaeaceae also exists in the Cucurbitaceae (cf. Fig. 99). We have already seen that every placental ridge has a more or less prominent bundle in its inner angle and this bundle has been interpreted above as the ventral strand. If this is so, we cannot escape the conclusion that the carpellary margins in the Cucurbitaceae proceed inward at least as far as the internal bundles and form the central core of the placental ridges. The possibility of the placental ridges being mere outgrowths from the carpellary margins is, therefore, ruled out.

Further, it may be argued that if the whole placental ridge is not an outgrowth, it may be only partly so. For instance, it may be claimed that the carpellary margins end near about the ventral strands and the structures curving outward across the middle of each chamber are merely placental outgrowths arising from them (cf. Fig. 100).

As against this, there is the third view put forward by Eichler in 1875 in his monumental book — *Blüthendiagramme* — and subsequently supported by Rendle (1925) and Judson (1929). According to this, the carpellary edges " meet in the centre of the ovary and then curve outward across the middle of each chamber, bifurcating near the circumference and bearing ovules on two incurving edges " (Rendle, 1925) (cf. Fig. 101).

The main point at issue, therefore, is with regard to the nature of the structures (shown hatched in Fig. 100) which curve outward and bear ovules, whether they are carpellary margins themselves or just outgrowths from them. This issue can be settled only in an arbitrary manner, and no useful purpose will be served by discussing it further.

Whatever may be the morphological nature of these ovule-bearing structures,

there is little doubt that the placental ridges in the Cucurbitaceae usually consist of two parts. There is the internal, more or less wedge-shaped, core which is formed by the septum and on either side of it there is the ovule-bearing region which is more or less fused with it. In Figs. 99-101 they are shown as distinct structures for the sake of clarity. The pulp within the cucurbit ovary, therefore, is partly septal and partly placental and not entirely placental as is described by certain taxonomists. Kerner and Oliver (1897), however, believe that it is the stalks of the ovules that fill the cavity of the ovary so completely that only small interstices are left between them.

PLACENTATION — It has already been pointed out (Puri, 1952b) that the position and composition of the placental strands are features of great significance in determining the mode of placentation. In typical axile placentation, for instance, these strands occur on the same radii as the carpellary dorsals and are formed by the fusion of two ventral bundles of the *same* carpel. In parietal placentation, on the other hand, they occur on radii alternating with those of the dorsals, i.e. on septal radii, and are composed of two ventrals belonging to two *different* carpels (cf. Puri, 1952b). In all the cucurbits examined here (with the possible exception of *Fevillea*), it will be seen that the placental strands occur on septal radii and never opposite the dorsals. It is quite apparent, therefore, that anatomically the placentation is always parietal. The fact that in certain cases the fruit becomes multilocular is due to secondary fusion of the adjacent placental ridges.

The condition in *Fevillea*, however, is noteworthy. Here, as will be recalled, there is a single central bundle which traverses as such almost throughout the length of the ovary. It is only in the upper region that it splits into three branches. This is clearly the anatomy of axile placentation, for here all the six ventral bundles of three carpels have apparently fused together. In *Fevillea*, therefore, the placentation, both in appearance and in anatomy, is axile. Baillon (1888, p. 377) also speaks of axile placentation when he describes the *Fevillea* series.

SOME EVOLUTIONARY TRENDS IN THE CUCURBITACEAE — Reduction in the number of carpels appears to be a well-established evolutionary tendency in the Cucurbitaceae. In *Apodanthera*, which is ranked amongst the most primitive genera, there are still five well-developed carpels. In the tribe Cucurbiteae this number is persistently three, but in the Sicyoideae and the Cyclanthereae, which are placed highest by Müller and Pax (1894), there is a marked tendency towards reduction in the number of placentae which results in pseudomonomery. In Sicyoideae, although the number of carpels cannot be determined in the ovary region, unmistakable evidence of the presence of three carpels exists in the style, where there are three carpellary dorsals supplying the three stigmas. In *Cyclanthera*, which is believed to be the most highly evolved genus, it is possible that a true monocarpellary condition has been achieved but this could not be definitely determined. Kratzer (1918) has figured and described some interesting cases of reduction in the number of carpels in *Echinocystis* and *Cyclanthera*.

Reduction in the number of carpels has been followed by a corresponding reduction in the number of ovules. This tendency has reached its climax in the tribe Sicyoideae which shows a uniovulate condition. In *Bryonopsis* although there are only about six ovules, the number of ovular traces that pass out into the ovule-bearing regions is more than six. In view of this it is possible that in the ancestral forms there may have been more ovules than are present in the modern species. Again, it has been pointed out that in *Sicyos* the single ovule receives its vascular supply from three different sources. Here too it appears logical to conclude that bundles which should ordinarily have gone to three different ovules enter the single ovule present. A similar situation in certain Cyperaceae has also been interpreted in the same manner (Blaser, 1941).

Another important evolutionary tendency is with respect to placentation. As mentioned above, in *Fevillea* the placentation is typically axile, both in

anatomy and appearance. In all taxonomic treatments (Baillon, 1888; Müller & Pax, 1894) this genus is considered to to be most primitive of all cucurbits. If this is correct, it seems logical to conclude that in the Cucurbitaceae there has occurred a change from axile to parietal placentation (cf. Puri, 1952b). This is seen in *Fevillea* itself where, after the division of the central bundle into three, the ovary becomes unilocular in its upper sterile region (Fig. 11). In Cucurbiteae, the largest tribe, although the placentation may *appear* to be axile owing to secondary fusion of adjacent placental ridges, its anatomy, particularly in the upper region, is always that of parietal placentation. In *Citrullus* the occurrence of a central stele also seems to indicate that the ancestors may have had axile placentation. In *Echinocystis* the placentation is axile at the extreme base but becomes parietal in the upper region. In the most highly evolved tribe, Cyclanthereae, the parietal condition is well established from the very beginning.

Summary

The paper deals with the structure and vascular supply of the cucurbit gynaeceum, with particular reference to placentation. Some thirty species have been examined and ten of them, representing the five tribes into which the Cucurbitaceae is divided, have been described in detail.

The gynaeceum, in different genera, consists of 2-5 carpels, three being the commonest number. It is doubtful if a truly monocarpellary condition has yet been achieved in the family although pseudomonomery is well established, particularly in the tribes Sicyoideae and Cyclanthereae. The ovular supply is obtained differently in different species, and this fact has been interpreted to indicate that the placentation in the family is still in a ' fluid ' condition.

Every one of the placental ridges contains one prominent inverted bundle, the internal bundle, in its inner angle. This is interpreted as the ventral strand and its inverse orientation has been explained in the same way as that of the ventral strands in the Passifloraceae, Moringaceae and other rhoeadalean families.

Attention is directed to the problem whether the placentae are formed entirely by carpellary margins or whether they are distinctive structures arising as outgrowths from them. The placental ridges are interpreted as " compound " structures, consisting partly of septal tissue and partly of placental tissue. The pulp in the mature cucurbit fruit, therefore, is not entirely placental, as has generally been believed.

The placentation is considered to be parietal. Additional support for such an inference is obtained from the fact that the ventral strands always occur on septal radii.

Finally attention is drawn to some evolutionary trends in the gynaeceum of the Cucurbitaceae. Besides the usual reduction in the number of carpels and the number of ovules, evolution in the cucurbit gynaeceum seems to have progressed from axile placentation to parietal placentation.

The author is thankful to Mr. M. L. Garg[7], Research Scholar, for inking the figures; to Mr. H. P. Sharma for drawing the theoretical diagrams; to several other friends who have helped him in collecting the material; and to the Government of Uttar Pradesh for providing some technical assistance and financial help towards the completion of this work.

7. The author regrets to announce the untimely death of Mr. Garg on May 25, 1954.

Literature Cited

Arber, A. 1938. Studies in flower structure. V. On the gynaeceum of *Papaver* and other related genera. Ann. Bot. **2**: 649-663.

Bailey, I. W. & Nast, C. G. 1945. The comparative morphology of the Winteraceae. VII. Summary and conclusions. J. Arnold Arb. **26**: 37-47.

Baillon, H. 1888. " The natural history of plants." Vol. VIII. (Eng. Transl. London.)

Blaser, H. W. 1941. Studies in the morpho-

logy of the Cyperaceae. 1. Morphology of the flowers. B. Rynohosporoid genera. American J. Bot. **28:** 832-838.

Bonnier, G. & Du Sablon, L. D. 1919. "Cours de Botanique. Phanerogames." Paris.

Brongniart, A. 1827. Mémoire sur la génération et developpement de l'embryon dans les vegetaux phanerogames. Ann. Sci. Nat. Bot. **12:** 14-53, 145-172, 225-296.

Devi, Shanti 1952. Studies in the order Parietales. III. Vascular anatomy of the flower of *Carica papaya* L. with special reference to the structure of the gynaeceum. Proc. Indian Acad. Sci. B. **36:** 59-69.

Eames, A. J. & MacDaniels, L. H. 1947. "An Introduction to Plant Anatomy." 2nd Ed. New York.

Eggers, O. 1935. Über die morphologische Bedeutung des Leitbundelverlaufes in der Blüten der Rhoeadalen and über des Diagramm der Cruciferen und Capparidaceen. Planta **24:** 14-58.

Eichier, A. W. 1875. "Blüthendiagramme." Vol. I.

Gaertner, J. 1791. "De fructibus et seminibus plantarum." (Quoted from Kirkwood, 1905.)

Grew, N. 1682. "The Anatomy of Plants with an idea of a Philosophical History of Plants." London.

Haines, H. H. 1922. "The Botany of Bihar and Orissa." Vol. III. London.

Heinig, K. H. 1951. Studies in the floral morphology of the Thymelaeaceae. American J. Bot. **38:** 113-132.

Hooker, J. D. 1897. "The Flora of British India." Vol. II. London.

Judson, J. E. 1929. The morphology and vascular anatomy of the pistillate flowers of the cucumber. American J. Bot. **16:** 69-86.

Kerner, A. & Oliver, F. W. 1897. "The Natural History of Plants." Vol. VI. London.

Kirkwood, J. E. 1905. The comparative embryology of the Cucurbitaceae. Bull. New York. Bot. Gard. **3:** 313-402.

Kratzer, J. 1918. Die verwandtschatlichen Beziehungen der Cucurbitaceen auf Grund ihrer Samenentwicklung mit spezieller Berucksichtigung der Caricaceen, Passifloraceen Aristolochiaceen und Loasaceen. Flora **110:** 275-343.

Le Maout, Emm. & Decaisne, J. 1876. "A General System of Botany, Descriptive and Analytical." Eng. transl. London.

Lindley, J. 1853. "The Vegetable Kingdom." 3rd Ed. London.

Mirbel, C. F. B. 1815. "Elements de Physiologie Vegetable." (Quoted from Kirkwood, 1905.)

Müller, E. G. O. & Pax, U. F. 1894. Cucurbitaceae. In Engler and Prantl's "Die naturlichen Pflanzenfamilien" **4:** 1-39.

Payer, J. B. & Baillon, H. 1872. "Leçons sur les Families naturelles des plants." Paris.

Prantl, K. 1897. "An Elementary Text-book of Botany." Engl. Transl. London.

Puri, V. 1951. Rôle of floral anatomy in the solution of morphological problems. Bot. Rev. **17:** 271-353.

— 1952a. "The Flower 3. Placentation in the Rhoeadalean Flower." Agra Univ. Exten. Lecture, Agra.

— 1952b. Placentation in Angiosperms. Bot. Rev. **18:** 603-651.

Rendle, A. B. 1925. "The Classification of Flowering Plants." Vol. II. Cambridge.

Saint-Hilaire, A. de 1847. "Leçons de botanique." Paris.

Saunders, E. R. 1936. Some morphological problems presented by the flower in the Nymphaeaceae. J. Bot. **74:** 217-221.

Schleiden, M. J. 1849. "Principles of Scientific Botany." Engl. transl. London.

Trimen, H. 1894. "A Hand-book to the Flora of Ceylon." Vol. II. London.

Warming, M. E. & Potter, M. C. 1932. "A Hand-book of Systematic Botany." 2nd Ed. London.

Wilkinson, A. M. 1944. Floral anatomy of some species of *Cornus*. Bull. Torrey Bot. Cl. **71:** 276-301.

Willis, J. C. 1948. "A Dictionary of the Flowering Plants and Ferns." Cambridge.

THE SIGNIFICANCE OF THE HISTOCHEMICAL LOCALIZATION OF QUINONES IN THE DIFFERENTIATION OF PLANT TISSUES

D. S. VAN FLEET

Department of Botany, University of Missouri, Columbia, Missouri, U.S.A.

Introduction

Naphthoquinones and anthraquinones may be used as natural intracellular oxidation-reduction indicators of reducing or oxidizing intensity in comprehensive studies of tissues at the level of cell division and differentiation. Preliminary trials revealed that the state of the relative oxidation-reduction of the quinones may be used to determine stages of histogenesis from division through differentiation and maturation (Van Fleet, 1954). A more extensive survey was then conducted of the oxidation-reduction potentials of many quinones and of their oxidized or reduced condition in sequential stages of histogenesis. The goal in this work was to establish the quinones as indicators of " reducing intensity " rather than as indicators of oxidation-reduction potential in the thermodynamic equation (cf. Cohen, 1933). The assumption was made that these internal indicators contribute to and are a part of the first signs of differentiation, and that they give some inference as to the free energy level of various tissues as they differentiate.

Quinones have an inhibitory and catalytic action on certain respiratory processes (Ball, Anfinson & Cooper, 1947; Fieser, 1948; Gutzman-Barron, 1951; Friedheim, 1934; Schopfer & Grob, 1949), and quinones have been demonstrated to inhibit cell division (Hoffman-Ostenhof, 1947, 1950; Reed, 1949; Levan & Tjio, 1948; Huber, 1947; Meier & Allgöwer, 1947; Nybom & Knutsson, 1947). The fungicidal and bactericidal action of various quinones has been related to their cellular reactivity as inhibitors of essential enzymes and of cell division (review by McNew & Burchfield, 1951).

The importance of quinones as enzyme inhibitors and as poising agents in oxidation-reduction has been impressively reiterated in the literature, but the emphasis has been on the intercalation of diverse quinones into recognized metabolic systems or organisms. There has not been an examination or interpretation of the histochemical localization of quinones and their capacity as redox indicators and as causal agents in histogenesis in vascular plants.

Materials and Methods

Histochemical Localization — The distribution of the reduced and colorless precursors of the benzoquinones, naphthoquinones and anthraquinones was determined by applying potassium ferricyanide (0·002 M) as an oxidant to freezing microtome or free-hand sections. In many cases an oxidant was not necessary, exposure of the sections to air in cutting was sufficient, with injury and the attendant activation of phenolases and rise in oxidation potential, to reveal the strongly colored and oxidized form of the quinone.

The nitroso colored derivatives of phenols and quinones were found to be the best method of obtaining the pattern of distribution and the points at which these substances first appear. The formation of nitrosophenols and the tautomeric quinone monoximes was induced by nitrous acid reaction with the phenols (Hickinbottom, 1940; Reeve, 1951) and by the action of nitrous acid or hydroxylamine hydrochloride on the quinones. By adding potassium hydroxide (5 per cent) the highly colored salt of the nitroso or oxime derivatives was obtained. Other color reactions, particularly of the naph-

thoquinones, were used. Some naphthoquinones gave a red insoluble product with nickel acetate (Brissemoret & Combes, 1907). Copper acetate and ferric salts have been used for color reactions of naphthoquinones, but with most naphtho and anthraquinones reproducible results were not obtained. Plants chosen for the histochemical localization studies were from families of plants known to contain quinones, and many of the plants are listed in the extensive literature on quinones.

In measuring the oxidation or reduction color changes of a quinone in a tissue, a microspectrophotometer was used to obtain the percentage of transmission at 10 mμ intervals (from 400 to 700 millimicrons) in the visible spectrum. The microspectrophotometer used was constructed by the writer as a modification of a spectrophotometer made by Safford and Westneat (1953) in which a wedge interference filter (Bausch & Lomb) was employed to obtain monochromatic light.

Oxidation-reduction Methods — By reason of the absence of an equilibrium system and of the heterogeneous redox content of the cell it is not possible to obtain an oxidation-reduction potential in the sense of a true thermodynamic equilibrium. It is recognized, however, that the reducing or oxidizing capacity or intensity of individual tissues may be obtained. Somewhat accordant and reproducible measurements may be obtained when redox electrodes are placed in a suspension of cells (Canaan, Cohen & Clark, 1926) or even directly into some tissues (Van Fleet, 1954). Redox indicators injected into cells and tissues also give significant correlations with growth phases even though there is inherent error in damage and poising effects. In order to avoid the errors of electrode and indicator application, one may follow the redox behavior of natural internal indicators like the quinones. The only practical method for studying the relative reducing or oxidizing intensity of many of the fundamental tissues is to follow colorimetrically the rate of inception of the reduced and finally the oxidized form of a naphthoquinone.

In the oxidation-reduction measurements reported here standard potentiometric methods were used (Clark *et al.*, 1928; Hewitt, 1951; Beckman Bulletin 99-D, 1951). The performance and potentials of the calomel reference electrodes were checked against phthalate and quinhydrone buffers. Several platinum electrodes were used in each system to avoid error and two glass electrodes were used to determine *p*H and *r*H.

Solutions of quinones extracted from the plant in alcohol and water were placed in a closed electrode chamber and nitrogen was bubbled through the system to remove oxygen and to stir the solutions. Nitrogen was first bubbled through Fieser's solution (alkaline sodium hydrosulfite and anthraquinone sulfonate) to remove traces of oxygen. Oxidation-reduction titrations were made to determine the potential (Eh) at the point of development or loss of color. Sodium hydrosulfite (6×10^{-4} N) was used as a reductant, reductions were also carried out with hydrogen gas bubbled through platinized asbestos and potassium ferrocyanide (0·01 N) was used as the oxidant. Phosphate or barbiturate *p*H buffers were used in all titrations. Fresh sections were observed under the binocular dissecting microscope and/or under the binocular microscope at the same time electrodes were placed in the tissue suspension in either a reducing or oxidizing medium.

From the indirect redox behavior of quinones *in vitro* and direct observation of their oxidation-reduction *in vivo*, estimates were made of the redox range of the quinones and of the probable redox range of the cells in which they occurred. In all cases, the redox potentials obtained were in a heterogeneous system and the results are presented as reducing or oxidizing intensities of primary significance, but not as potentials comparable to those obtained under conditions of an artificial and noncellular equilibrium. Within the limitations of the general methods outlined above, the following results are presented as one of the few practical means of fixing the boundaries of reducing or oxidizing intensity of quinone systems in plant tissues at the level of tissue differentiation.

Results

General Histochemical Localization — Quinones occur as the reduced and colorless hydroquinones, hydronaphthoquinones and hydroanthraquinones in the differentiating endodermis, hypodermis, epidermis, outer cortex, xylem and some of the phloem elements. The heaviest localization in roots was found to be in the endodermis (Figs. 1-3). Cambium of stems and roots contained reduced quinones; the visible oxidized quinones appeared on cutting sections and adding oxidants. Derivatives of the cambium had visible quinones increasing in quantity with progression in differentiation and age. Ray cells had the highest amount of oxidized quinones with lesser amounts in the xylem and phloem elements.

Localization of hydronaphthoquinone in the cambium of *Juglans nigra* was quite clear and the reduced hydronaphthoquinone readily oxidized on exposing the cambium in fresh sections. Anthraquinones were found to be localized in ray cells and in the ray initials of the cambium of *Rheum rhaponticum* and other members of the *Rubiaceae* and *Rhamnaceae*. Where a skip in the mitotic cycle of ray initials occurred, as previously described (Van Fleet, 1954), there was a heavy localization of naphthoquinones and anthraquinones.

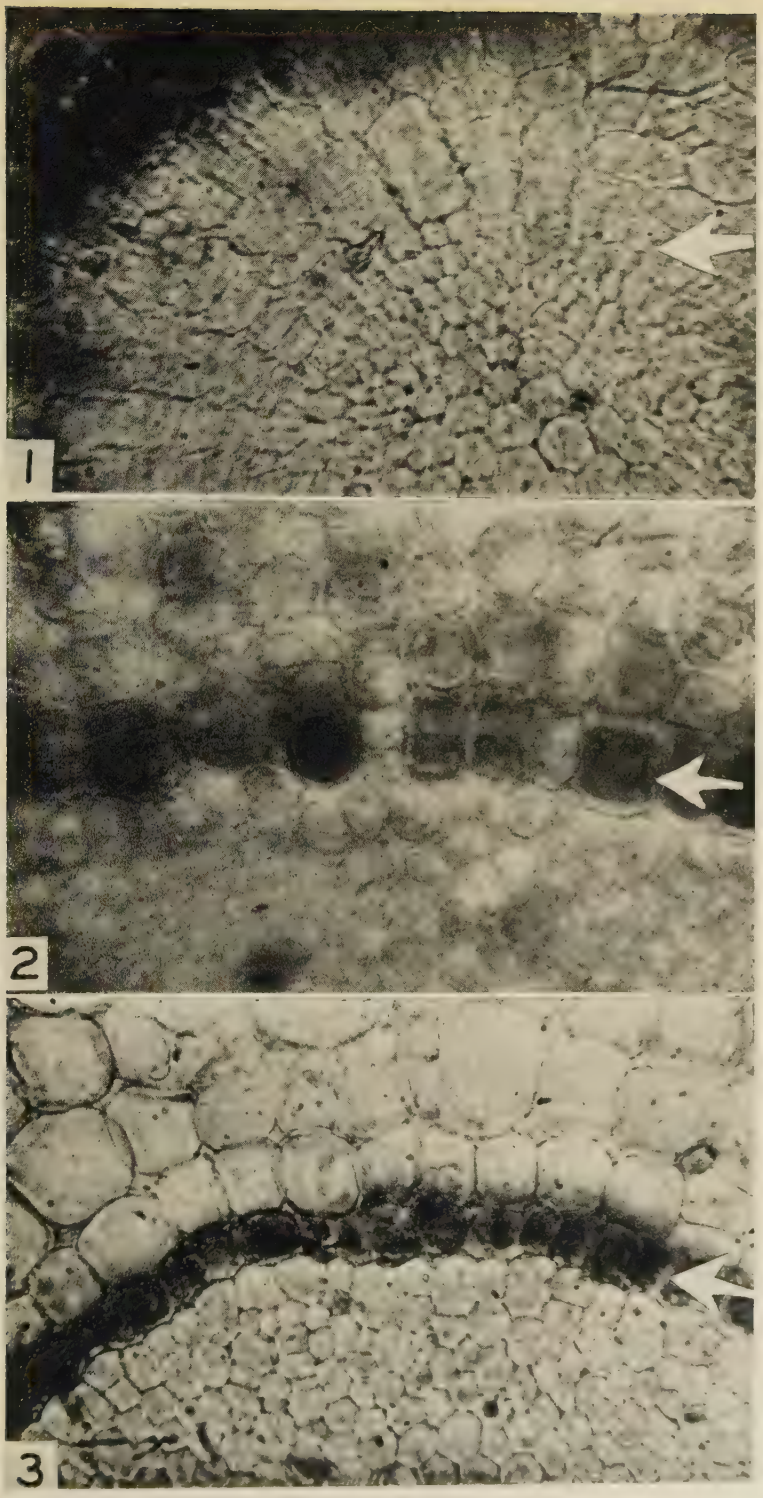
Anthraquinones and naphthaquinones were found in non-dividing cells whereas their precursors, hydronaphthoquinones and hydroanthraquinones, were found in dividing cambium. Cambial tissue exposed to the air became positive in redox potential, as determined by direct application of electrodes and by suspensions of cell scrapings in electrode chambers. With a loss in reducing intensity in the cambium there was a rise of as much as 350 millivolts to high positive levels and at the same time the oxidized and colored form of the quinones became visible. These changes in color and potential were localized and definite and were not capricious or random.

Localization of Hydronaphthoquinones and Histogenesis in Roots — Naphthoquinone distribution was examined in *Juglans nigra, Tecoma radicans, Medicago sativa, Celastrus scandens, Impatiens capensis, Impatiens pallida* and *Impatiens balsamina* as representatives of several families (Juglandaceae, Balsaminaceae, Bignoniacea) notable for their high content of naphthoquinones. The localization and redox reversibility of the naphthoquinones could be readily demonstrated in all of these plants, but the most inferential and consistently reproducible results were attained in *Impatiens* species and in particular in white-flowered* *Impatiens balsamina*. The white-flowered balsam was free of anthocyanin which sometimes interferes with localization studies in the roots of *I. pallida, I. bicolor* and anthocyanin-bearing forms of *I. balsamina*.

In serial sections of the meristem of root of *Impatiens* it was found that there is a heavy localization of hydronaphthoquinone in the endodermis. At the stage where the endodermis is still dividing, to give rise to the cortex (see Williams, 1947), the hydronaphthoquinone was not present (Fig. 1). At the point where divisions cease and the endodermis begins to differentiate, large amounts of hydronaphthoquinone were present (Figs. 2, 3). The hydronaphthoquinone could be readily induced to oxidize to the colored naphthoquinone. The general color reactions of this naphthoquinone *in situ* were similar to those obtained by Little, Sproston and Foote (1948) who identified the naphthoquinone extracted from *Impatiens balsamina* as a 2-methoxy-1, 4-naphthoquinone.

At the level of cell division in the origin of the cortex, the reversible quinone system was not detectable, but at the point where cell division stopped quinones were readily identified (Figs. 1-3). From the relative redox behavior of this naphthoquinone one may adduce that at the end of the division phase for the endodermis, the Eh of the cells at *p*H 7 is at least −0·090 to −0·120 V and duplicating determinations gave an *r*H of 10 to 11. In old endodermal cells remote from the division zone,

*Seed of white-flowered *Impatiens balsamina* was generously supplied by N. V. Sluis en Groots and grateful acknowledgement is made of the interest and help of N. Sluis, of Sluis & Groot of America, in obtaining this seed.



FIGS. 1-3 — Transections of root tip of *Impatiens balsamina* showing successive stages in differentiation of naphthoquinone in the endodermis from the level of cell division through early differentiation. × 440. Fig. 1. Arrow points to the endodermis where it is still dividing and the naphthoquinone has not been developed. Fig. 2. Late stages in division of the endodermis with the beginning of naphthoquinone formation. Fig. 3. Fully developed naphthoquinone localization in the endodermis at the end of the division phase and the beginning of full differentiation.

where occasionally there are visible and oxidized quinones, the apparent Eh was at least −0·020 to +0·100 V or higher (rH, 13·5 to 17).

Naphthoquinones in the Cambium and its Derivatives — Hydronaphthoquinones in the cambium of *Celastrus scandens* and *Juglans nigra* were oxidized to the colored naphthoquinone when the tissue was exposed to air. When sections were cut under nitrogen and held in sodium hydrosulfite (6×10^{-4} N), the hydronaphthoquinone remained in the colorless reduced state. Older xylem and phloem cells, and in particular the ray cells, contained oxidized naphthoquinones. On exposure to air, oxygen or ferricyanide, most of the cells in stems and roots showed the development of naphthoquinone. Reversible reduction and reoxidation was easily demonstrable with dilute hydrosulfite and ferricyanide, or with hydrogen bubbled through platinized asbestos and reoxidation by oxygen from the air.

Extracts of stems and roots of *Juglans nigra* were near the completely colorless reduction point at Eh +0·098 V at *p*H 7 and were in the oxidized and full color form at Eh +0·194 V at *p*H 7. When electrodes were placed in the cambium of a one year old twig with a barbiturate buffer at *p*H 7·4, there was no color at Eh −0·040 V with a beginning of coloration at Eh +0·030 V and full color at approximately Eh +0·090. Older twigs gave consistently higher potentials, but all the results were somewhat variable because of the rapid changes in the tissue and the slow adjustment of the electrodes. These results suggest that the cambium has an rH as low as 12 or lower whereas some of the mature ray cells and the cells of the xylem and phloem, containing visible naphthoquinones at the moment of sectioning, have an rH of 17 or higher.

Naphthoquinone in *Medicago sativa* was found to be localized in the cork tissue of the sub-aerial stem. The internal cork in the below-ground stem, derived from a meristematic endodermis, developed naphthoquinone at the time of differentiation. The meristematic endodermis did not contain any visible reduced or oxidized menadione (the vitamin K naphthoquinone in *Medicago*). Only the oldest fully differentiated daughter cells contained the reduced hydronaphthoquinone, and only the dead cork cells had any pigmentation that showed redox reversibility and gave the general color tests for a naphthoquinone.

Celastrol, the alkyl hydronaphthoquinone in *Celastrus scandens*, was found to be localized in the oxidized form in the oldest phloem elements of the bark of the root. Differentiating ray cells, young phloem next to the cambium and the cambium did not have either the reduced or the oxidized celastrol.

Lapachol, the naphthoquinone in *Tecoma radicans*, gave a red color product with ammonia or alcoholic potassium hydroxide. It was found to be in the oxidized condition in the oldest phloem elements, the mature xylem and xylem ray cells, and it was in the reduced state in the differentiating xylem elements. The reductant of lapachol is clearly present at the level of differentiation of the xylem. The yellow oxidant appeared when ferricyanide was added to the developing young xylem units, and in old roots the cambium apparently contained a small amount of the reduced lapachol. The yellow hydroxynaphthoquinone could be reversibly oxidized and reduced. Alcohol and water extracts of lapachol became colorless on reduction at an Eh of approximately −0·150 V at *p*H 7. Ball (1936) has determined the E_0^1 (potential at 50 per cent oxidation) of lapachol at various *p*H values; at *p*H 6·4 the E_0^1 was −0·134 V. The general rH range for lapachol must be roughly 9·5 to 11 and it is significant that the reduced form does not occur commonly in all tissues as do related hydroxynaphthoquinones having a higher potential. Lapachol with its lower point of reduction was found in the oxidized state only in young stems and roots.

Where the redox potential of a naphthoquinone was quite negative (rH 8-12), the oxidized and colored form was commonly found in the mature tissues and the reductant was rarely encountered. If the naphthoquinone had a higher reduction potential (rH 12-18), as in the naphthoquinones of the Juglandaceae, the reduced form was commonly found in all tissues

and the oxidized form only in old and apparently dead cells. These are significant observations and they suggest that naphthoquinone development may be correlated with the energy intensity in the dividing cells of the cambium and in the derived non-dividing cells of xylem and phloem.

When naphthoquinones were present in the oxidized and visible pigmented form, cell division had ceased. Pigmentation increased with age and with distance from the meristem, and the depth of pigmentation could be correlated with the stage of differentiation. The energy potential for the development of each tissue was reflected in the intensity of naphthoquinone localization. In general, the dividing cells appeared to contain less hydronaphthoquinones than the differentiating cells and the oxidized naphthoquinones were found only in mature cells, dead vessels, necrotic areas in the phloem and xylem and the oldest portions of rays.

The naphthoquinone in *Lithospermum canescens* was found to be in a bright red to brown oxidized form in localized groups of cells in the cortex of the root. The reduced form of the quinone could not be found in any cells of the plant. On reduction of the oxidized quinone a lightly colored product was formed. It was not possible to extract from the plant or detect in the tissue a reversible oxidation-reduction naphthoquinone. The characteristic light absorption of the oxidized quinone and its irreversible insoluble reduced form are shown in Fig. 4. The color of the reduced insoluble and irreversible product was difficult to define until the microspectrophotometer was used to obtain its absorption characteristics. Reversible naphthoquinones, in the tissue, gave color density characteristics like those shown in Fig. 5 for the naphthoquinone of *Juglans nigra.*

Localization of Anthraquinones — Anthraquinones in roots of *Rheum rhaponticum* were found to be in the oxidized form in the xylem and phloem ray parenchyma cells. The mixture of anthraquinones in rhubarb (emodin, isoemodin, chrysophanic acid, chrysarone, rhabarberone, etc.) obviated precise redox studies, but there were some consistent observations on localization and general oxidation of the anthraquinone complex. The anthraquinones were apparently not in the cambium in either the oxidized or the reduced condition, but the cambial ray initials contained the reduced form in young active roots. Cambial ray initials in old roots contained oxidized anthraquinones associated with a skip in the mitotic cycle in the cambium (Van Fleet, 1954).

Cambial ray initials did not divide regularly in older roots and where the ray initials became filled with oxidized anthraquinone the cells elongated, failed to divide further and lost their meristematic appearance as indicated by their form when compared with surrounding cambial cells. Titration of anthraquinone extracts showed complete reduction at about −0·040 V at *p*H 7·2 with hydrosulfite as the reducing agent. Reoxidation by ferricyanide occurred at +0·046 V. These anthraquinones revealed a potential change of at least 100 mV in the meta-

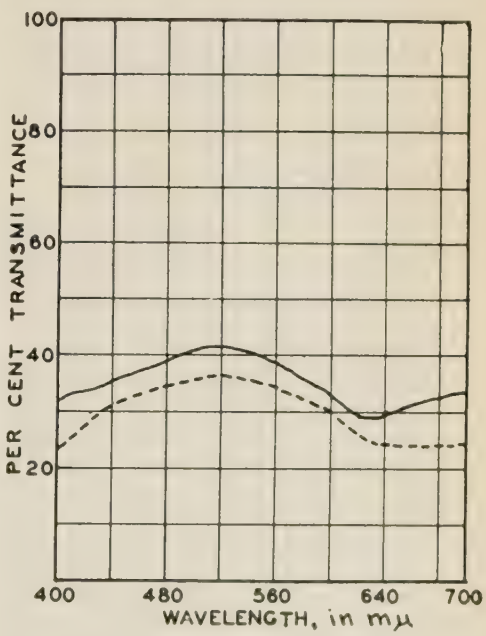


Fig. 4 — Curves for the irreversible naphthoquinone in the cortex of *Lithospermum canescens.* Solid line is the transmission curve of the completely reduced compound. Dotted line is the curve for the oxidized naphthoquinone. Both curves obtained from the same group of cortical cells.

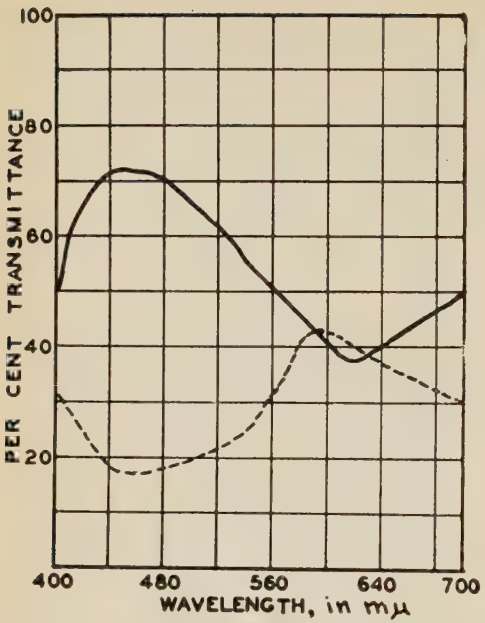


FIG. 5 — Curves showing typical spectral transmission of a naphthoquinone in *Juglans nigra*. Solid line is the approximately completely reduced quinone. Dotted line is for the fully oxidized quinone. Both curves from the same group of cambial cells. Spectral response of the quinone outside the tissue is not like the curves shown here.

bolic shift from division to differentiation phase.

Normal cambial cells and differentiating xylem and phloem cells reduced tetrazolium salts to formazans, but the older cambial ray initials containing quinones did not reduce either tetrazolium salts or the reversible redox indicators (methylene blue and 2, 6-dichlorophenol indophenol).

Anthraquinone in *Cephalanthus occidentalis* was found in the reduced form in the fresh cambium, but became reversibly oxidized on scraping the cambium or exposing it in making sections. Sections made under nitrogen oxidized to the colored form slowly, if at all. In extracts and in sections the anthraquinone was reduced by hydrosulfite at *p*H 5·5 at an Eh of +0·092 V. Many phloem parenchyma cells and phloem sieve tubes contained the quinone in the oxidized form, the pigment present in these cells was redox reversible. In general a similar localization of anthraquinones was found in *Rhamnus cathartica, Berchemia scandens* and *Ceanothus americanus*.

Reduced hydroanthraquinone was found in the cambium of the root of *Ceanothus americanus*, the xylem contained the red anthraquinone. Injuries to the root, incident to the habitat of the plant, resulted in oxidation of hydroanthraquinone to anthraquinone in the cambium. The cambium did not divide further after anthraquinones formed in it (see Fig. 6). The oxidized anthraquinone indicated a loss of reduction capacity and the capacity of the cells to divide, and it is quite possible that the oxidized anthraquinones inhibited cell division by poising the redox level of the cell at a point unfavorable for cell division.

BENZOQUINONES — A survey of benzoquinones and their distribution has not been completed. Preliminary results on arbutin, a benzoquinone glucoside in *Pyrus communis* and *Arctostaphylos Uva-Ursi*, and related glucosides of indigo, parillin, etc., have indicated that B-glucosidase activation precedes the oxidation to the colored quinone (Van Fleet, 1952). These substances can be used in some cases to correlate redox level in the cell with differentiation.

The hydrolysis of parillin glucosides in *Smilax* and the subsequent oxidation intensity of phenol-phenolase systems is apparenty correlated with clonal type

FIG. 6 — Transection of stem of *Ceonothus scandens* showing anthraquinone in oxidized form on the side of the stem where division has been terminated. The singular one-sided action of the cambium has resulted in the irregular form shown in the photomicrograph and there is a correlated localization of oxidized anthraquinone on the inactive side of the stem. × 80.

and differentiation according to ecotypes. Phenol formation and oxidation in *Smilax* vary directly with latitude, altitude and general locale at the time of differentiation (Van Fleet, 1954).

Discussion

The development of hydroquinones is concomitant with a decline in cell division and apparently indicates a general loss in reducing capacity of meristem cells. The earliest stages of differentiation of cambial derivatives and of the cortex in the root are delineated by the development of hydronaphthoquinones and hydroanthraquinones. In cells with phenols and reduced quinones there must be a reducing capacity to hold these substances in a state of reduction.

There are three levels of growth and differentiation through which the quinones may be regarded as proceeding. The active division stage in which in most meristems there are only reduced quinones, a later stage of differentiation in which easily detectable and oxidizable quinones appear, and finally a late stage of full histological development in which quinone oxidation and polymerization in cells and cell walls may occur.

Quinone localization in some tissues, in particular the endodermis and the rays, may be a result of the localization of active phenolases. Oxidation of hydroquinones to quinones may be either enzymatic by phenolases or by a general redox change to positive and higher potentials. A loss of reduction capacity in the meristem is indicated in endodermal cells which cease their divisions at a critical and exact point in giving rise to the cortex. There is a general reduction capacity loss, as a result of loss of free energy in the cells, and simultaneously there is activation of phenol/quinone synthesis and associated enzyme activation.

From a fundamental histogenic viewpoint there is not any difference between redox capacity and the activation of phenolases. Enzymes determining the rate of development of phenol/quinone systems are probably functional only at the redox levels observed in their substrates. In any case, the equilibrium points of oxidation-reduction of the quinones would appear to be determinative in differentiation. Phenolases are catalysts which determine rate of reaction but not the inception, end-point or mid-point of a reversible redox system.

Quinone localization can be detected so early that it becomes apparent to the observer that these internal substance systems precede in time the formation of histologic and anatomical signs of differentiation. The endodermis may be detected, because of its high content of hydronaphthoquinones, at the stage where it is coming to the end of its division cycle in giving rise to daughter cortical cells. This precedes in position and time the development of quinone and fat condensation products in the formation of Casparian strips and suberin/quinone condensation products in endodermal walls.

Dihydroxyphenols, hydroxyhydronaphthoquinones and hydroxyhydroanthraquinones are apparently in the vacuolar sap in a reduced condition. With differentiation, age and necrosis there is a loss in reduction capacity and oxidation and polymerization of these components and they become aggregated in the vacuoles and in the cell walls. The quinones in the vacuoles appear to be under the direct influence of the reversible enzyme and redox systems of the protoplast and are not "outside" of the cell, but metabolically active substances are inactivated when they pass through the tonoplast into the vacuole (see Sutcliffe, 1953). In the case of the quinones there is a correlation with age and differentiation of the redox state of the quinones in the vacuoles and whether they are "outside" the cell is a hypothetical distinction. The content and anatomy of the vacuole is as intimately a part of histological development and distinction as the conformation of the cell wall or the presence or absence of any enzyme or substrate.

Quinoid compounds and their phenolic precursors may inhibit mitosis and enzymes by reacting with essential thiol groups (Hoffman-Ostenhof, 1950; Levan & Tjio, 1948 Loveless & Reveil, 1949). Quinones and phenols have been applied to whole tissue and found to act as mitotic inhibitors. Phenanthraquinone inhi-

bited growth of root and shoot cuttings of *Tradescantia* (Lehmann, 1947), and Reed (1947) found that cysteine hydrochloride as a hydrogen donor enabled cells to keep the dihydroxyphenols in a reduced state and mitosis was found to continue. Enzyme inhibition and mitotic inhibition by quinones may be through their protein binding action rather than by poising the redox balance of the cell at a level unfavorable for cell division (Fieser, 1948).

Histochemical localization of the phenols, naphthols, anthrols and their corresponding quinones is in tissues of an ancient origin. The causal localization of these formative systems may be in the survival value of their distribution in the dermal, perivascular and related tissues that are barriers to fungal, bacterial and other parasites. Active phenolases are found only to be markedly localized in the dermal, cork and perivascular tissues. Phenolases are present in all tissues but are activated only in old cells or with death or injury to the cells. Active B-glucosidase and phenolases, as well as their substrates, are localized in the living plant in the barrier dermal tissues (Van Fleet, 1948, 1950, 1952).

The development and localization of unsaturated fatty acids and their oxidation and deposition as semi-oxidized fatty acids in dermal tissues have been correlated with phenol-quinone systems (Van Fleet, 1952, 1954). Reversible phenol-quinone systems may act as pro-oxidants to accelerate oxidation and condensation and they may act with other substances as antioxidants. Accumulation of unsaturated fats and later their oxidation and condensation in the endodermis may be accounted for through the early causal localization of quinone systems. The anatomical and histogenic definition of the endodermis must, therefore, include a listing of the sequential steps in the localization of quinones and their function and molecular interaction with other systems to give the product called "endodermis" by the anatomist.

Summary

1. Intracellular reversible phenol-quinone systems were used to detect individual tissues at the level of differentiation. Oxidation-reduction changes were detected in separate tissues at the time of differentiation by observation of the state of reduction or oxidation of naphthoquinones and anthraquinones.

2. Potentials of extracted quinones were measured and estimates were then made of the relative potential or reducing capacity of the tissues in which the systems occurred.

3. Quinone oxidation products were found to be reduced by metabolites in undifferentiated cells, but with inception of differentiation there was apparently a loss in reducing capactiy and phenol-quinone systems were formed and quinones accumulated.

4. Quinones with a negative oxidation-reduction potential in the range rH 8-12 were found in the oxidized form only in mature tissues and the reduced form was rarely found and only in cambial tissue. Quinones with a higher potential in the range of rH 12-18 were present in reduced form in all tissues and the oxidized form was found only in old and dead cells. Quinone development was apparently correlated with definite levels of energy intensity in dividing cells and in their differentiating daughter cells.

5. Evidence was found that development of quinone systems was associated with cessation of cell division and the initiation of cell differentiation.

6. Cambial cells and recently derived xylem and phloem cells were relatively negative in oxidation-reduction potential, i.e. they showed a marked reduction capacity. Cambial tissue wounded and exposed to the air in sectioning lost its reducing capacity. Phenol-quinone systems reflected this change in potential and loss in reduction capacity by a color change.

7. The radiating lines of ray cells, and mature xylem and phloem elements, contained oxidized quinones, thus indicating a loss of reduction capacity. Older xylem and phloem cells had a lower quinone content than the rays.

8. Mitotic cessation in cambial ray initials was found to be associated with localization and development of oxidized quinones in the initials.

9. Cessation of division in the meristem (endodermis) that gives rise to cortex in roots was found to be associated with the inception, localization and development of phenol-quinone systems in the initials.

10. Quinones appear to be causal agents in inhibiting further cell division. Whether the quinones are in fact primary inhibitors of cell division, or whether they are merely internal indicators of a change from division to differentiation remains to be established.

11. There are at least three levels of histological development through which the quinones may be followed. The active division stage in which quinones are in the reduced state, if they are present at all, the beginning of differentiation in which easily detectable and oxidizable quinones appear, and a later stage of full histological development in which quinone oxidation and polymerization in cells and cell walls may occur.

12. That the phenol-quinone systems are an index to change in oxidation-reduction at the level of division and differentiation has been established, but their function as causal agents in differentiation must be explored further.

Literature Cited

Ball, E. G. 1936. Studies on oxidation-reduction. XXII. Lapachol, lomatiol, and related compounds. J. Biol. Chem. **114**: 649-654.

Ball, E. G., Anfinsen, C. B. & Cooper, O. 1947. The inhibitory action of naphthoquinones on respiratory processes. J. Biol. Chem. **168**: 257-270.

Beckman Bulletin 99-D. 1951. The measurement of oxidation-reduction potentials. Beckman Instruments Co. Pasedena, Calif.

Brissemoret, A. & Combes, R. 1907. Sur une reaction des oxyquinones. J. Pharm. et Chim. VI. **25**: 53-58.

Cannan, R. K., Cohen, B. & Clark, W. M. 1926. Studies on oxidation-reduction. X. Reduction potentials in cell suspensions. U.S. Publ. Health Reports, Supplement No. 55. 34 pp.

Clark, W. M. *et al.* 1928. Studies on oxidation-reduction. I-X. U.S. Publ. Health Reports.

Cohen, B. 1933. Reactions of oxidation-reduction indicators in biological material, and their interpretation. Symp. Quan. Biol. **1**: 195-204.

Fieser, L. F. 1948. Naphthoquinones as antimalarials and inhibitors of respiration. Biol. Antioxidants **3**: 24-32.

Friedheim, E. A. 1934. Natural reversible oxidation-reduction systems as accessory catalysts in respiration: Juglon and Lawson. Biochem. J. **28**: 180-188.

Gutzman-Barron, E. S. 1951. Thiol groups of biological importance. Adv. Enzymol. **11**: 201-266.

Hewitt, L. F. 1951. Oxidation-reduction potentials in bacteriology and biochemistry. 2nd. ed. London County Council.

Hickinbottom, W. J. 1940. "Reactions of Organic Compounds." Longmans Green & Co., New York.

Hoffmann-Ostenhof, O. 1947. Die Biochemie der Chinone. Experientia **3**: 176-184.

— 1950. Vorkommen und biochemisches Verhalten der Chinone. Fortsch. d. Chem. Org. Naturstoffe **6**: 154-241.

Huber, W. 1947. Über die antimitotische Wirkung von Naphthochinon und Phenanthrenchinon auf die Furchung von Tubifex. Rev. Suisse Zool. **54**: 61-154.

Lehmann, F. E. 1947. Chemische Beeinflussung der Zellteilung. Experientia **3**: 223-232.

Levan, A. & Tjio, J. H. 1948. Induction of chromosome fragmentation by phenols. Hereditas **34**: 453-483.

Little, J. E., Sproston, T. J. & Foote, M. F. 1948. Isolation and antifungal action of naturally occurring 2-methoxy-1, 4-naphthoquinone. J. Biol. Chem. **174**: 335-342.

Loveless, A. & Reveil, S. 1949. New evidence on the mode of action of "mitotic poisons". Nature (London) **164**: 938.

McNew, G. L. & Burchfield, H. P. 1951. Fungitoxicity and biological activity of quinones. Contrib. Boyce Thompson Inst. **16**: 357-374.

Meier, R. & Allgöwer, M. 1947. Zur Charakteriserung zellteilungswirksamer Substanzen an der Gewebekultur. Experientia **1**: 57-61.

Nybom, N. & Knutsson, B. 1947. Investigations on C-mitosis in *Allium cepa*. Hereditas **33**: 220-234.

Reed, H. S. 1947. A physiological study of boron deficiency in plants. Hilgardia **17**: 377-411.

— 1949. The action of quinones on mitosis. Experientia **5**: 237-239.

Reeve, R. M. 1951. Histochemical tests for polyphenols in plant tissues. Stain Technology **26-27**: 91-96.

Safford, H. W. & Westneat. 1953. An inexpensive easily constructed spectrophotometer J. Chem. Educ. **30**: 343-347

Schopfer, W. H. & Grob, E. B. 1949. Über den Einfluss von 1, 4 Naphtochinonderivatin mit Vitamin K — oder Anti-Vitamin K — Character auf Urease. Helv. Chim. Acta **32**: 829-838.

Sutcliffe, J. F. 1953. Ion Secretion in Plants. International Rev. Cytology II. **2**: 179-200.

Van Fleet, D. S. 1948. Cortical gradients and patterns in vascular plants. American J. Bot. **35**: 219-227.

— 1950. The cell forms, and their common substance reactions, in the parenchymal-vascular boundary. Bull. Torrey. Bot. Cl. **77**: 340-353.

— 1952. The histochemical localization of enzymes in vascular plants. Bot. Rev. **18**: 354-398.

— 1954. VI. Cell and tissue differentiation in relation to growth (plants). Dynamics of growth processes." p. 111-129. Princeton Univ. Press.

Williams, B. 1947. The structure of the meristematic root tip and origin of the primary tissues in the roots of vascular plants. American J. Bot. **34**: 455-462.

THE GAMETOPHYTE OF *DIDYMOCHLAENA SINUATA* DESV.

ALMA G. STOKEY & LENETTE R. ATKINSON

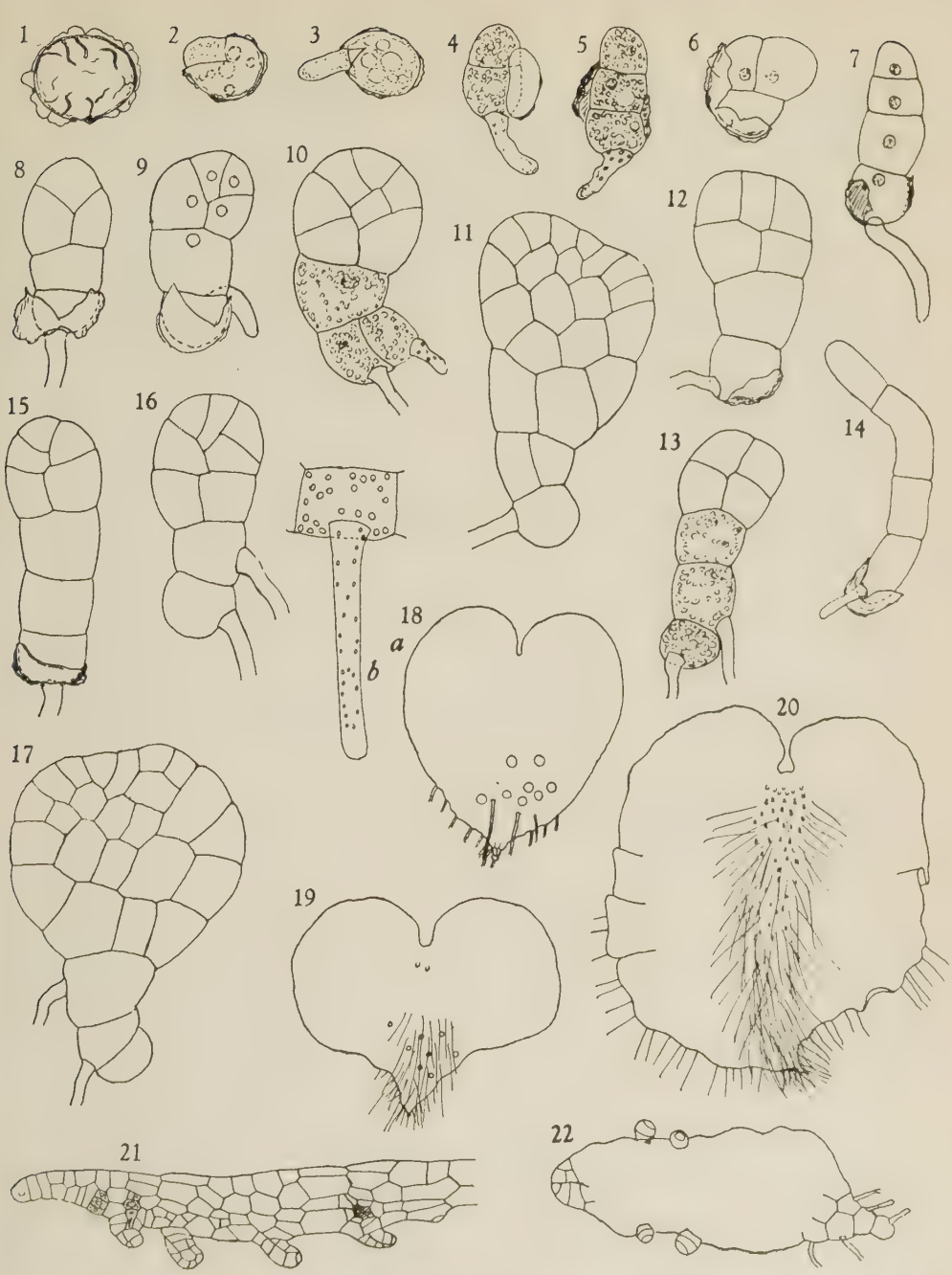
Mount Holyoke College, South Hadley, Mass., U.S.A. & Amherst, Mass., U.S.A.

Didymochlaena is a pantropic genus, usually regarded as consisting of a single polymorphic species, given by Copeland (1947) as *D. sinuata* Desv.; this is listed in Christensen's Index (1906) as *D. truncatula* (Sw.) J. Sm. We are indebted for the spores from which we made our cultures to the kindness of Dr. Walter Robyns, Director, Jardin Botanique de l'Etat, Brussels, where they were collected on May 27, 1953. Cultures were made on June 1, on distilled water, on porous clay crock resting on peat, and on peat.

The spores of *D. sinuata* are bilateral, brown and have a perispore (Fig. 1); oil drops are plainly visible; they average 36 × 29 μ in size. Germination occurred on water in 7 days; the spore coat breaks irregularly and the layers may separate. The faintly greenish prothallial cell may protrude first, or the rhizoid (Figs. 2, 3). Chloroplasts soon appear (Fig. 4) and a filament of several cells develops (Figs. 5, 7). Under favourable growth conditions, the filament consists of short cells above a green, more or less bulbous basal cell; if the culture is crowded, the slowly growing filaments consist of long cells (Fig. 14), but in our cultures we found no long filaments. Irregularities of growth in the early stages are not common, but a few thalli were found which were plate-like at this stage (Fig. 6) or had formed short branches. An apical cell may be formed early by oblique divisions, usually in the third or fourth cell of the filament (Figs. 8, 9, 15, 16); or the early divisions of the plate may be longitudinal and transverse (Figs. 12, 13) with a delay in the formation of the apical cell. Such variation is common in the higher ferns. The basal cell may undergo longitudinal division (Fig. 10). The apical initial continues activity for a varying number of divisions (Figs. 11, 17) before it is replaced by a marginal meristem.

The cordate form of the thallus was attained in 6 to 7 weeks, and antheridia were found on thalli before they were 7 weeks old (Fig. 18*a*). The thallus at maturity is cordate or reniform. Archegonia appeared at 11 weeks on gametophytes, some of which had already borne antheridia (Fig. 19). If growth is not checked by fertilization or other causes, the thallus elongates and continues to bear archegonia but not antheridia (Fig. 20).

A rhizoid is regularly developed on the basal cell (Figs. 4, 5, 7) and they may be



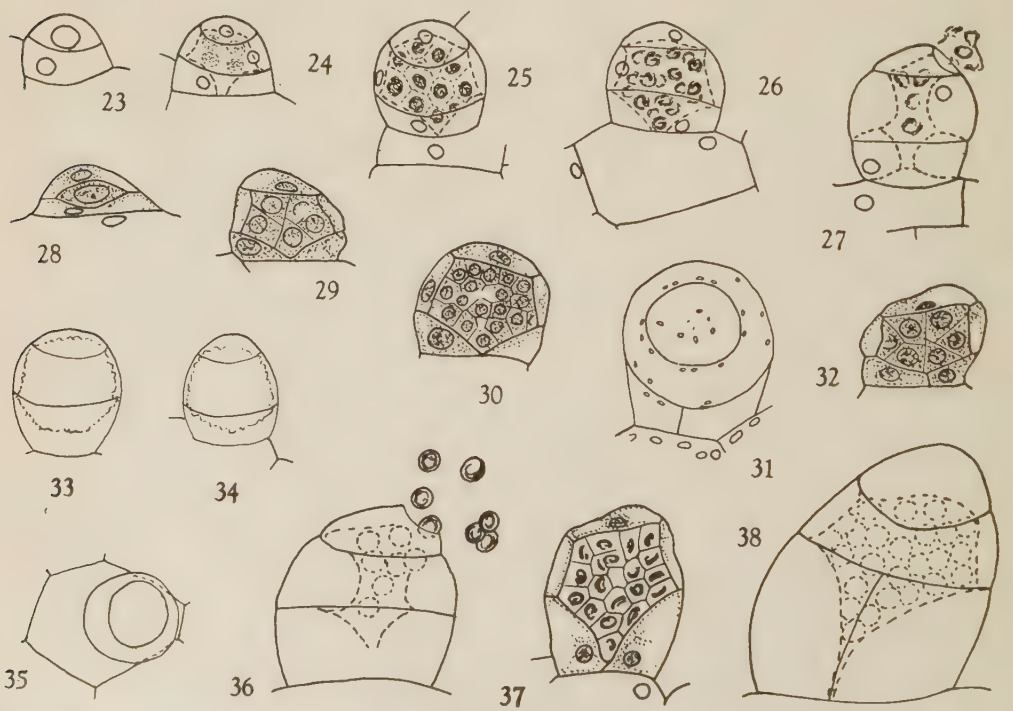
FIGS. 1-22 — Fig. 1. Spore. × 350. Figs. 2, 3. Prothallus, 7 days. × 225. Fig. 4. 10 days. × 225. Figs. 5, 6. 14 days. × 225. Fig. 7. 13 days. × 190. Figs. 8, 9. 20 days. × 225. Fig. 10. 24 days. × 225. Fig. 11. 25 days. × 190. Fig. 12. 22 days. × 225. Fig. 13. 25 days. × 225. Fig. 14. 10 weeks, crowded culture. × 225. Fig. 15. 24 days. × 225. Fig. 16. 38 days. × 225. Fig. 17. 38 days. × 190. Fig. 18*a*, *b*. *a*, 7 weeks with antheridia; *b*, marginal rhizoid from *a* with chloroplasts. *a*. × 30. *b*. × 225. Fig. 19. Thallus, 7 weeks, with 2 archegonia and 8 antheridia. × 10. Fig. 20. Thallus, 5½ months. × 10. Fig. 21. L.S. thallus. × 75. Fig. 22. 10 weeks, irregular thallus with antheridia. × 67.

borne on other cells of the filament (Figs. 13, 16). The rhizoids are usually colourless until the thallus is 6 or 7 weeks old, but on older gametophytes they have a faintly brownish tinge; they are soft in texture and those on old gametophytes are easily tangled. The rhizoids on young prothalli usually contain chloroplasts and the habit may persist for some weeks (Fig. 18*a*, *b*), much longer than is commonly the case except in primitive ferns. On gametophytes 6 or 7 weeks old chloroplasts may be found not only at the base of rhizoids but in the middle or even close to the tip. At this stage and often until a much later period, the rhizoids may be borne chiefly on the marginal cells; in the mature stages rhizoids are more abundant on the ventral cells (Fig. 20).

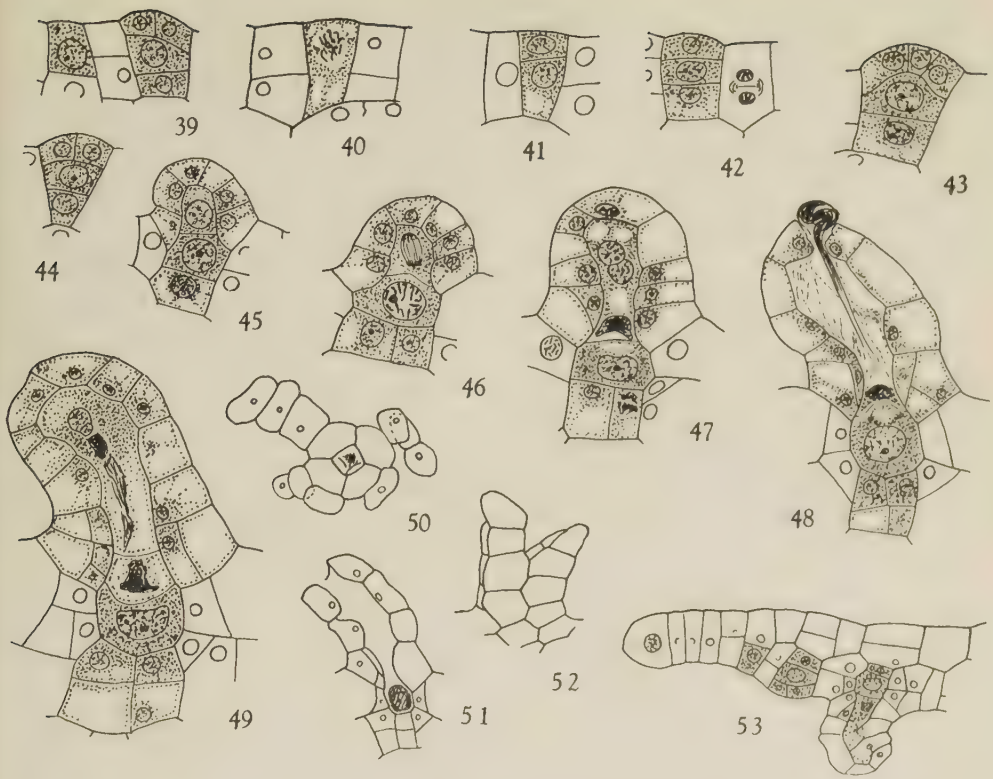
The thallus in our cultures was not thick and did not attain more than 5 or 6 cells in thickness (Fig. 21). The gametophyte of *D. sinuata* does not bear hairs at any stage.

Antheridium

The antheridia developed on the ventral surface of cordate thalli or on the surface and margins of irregular plates (Fig. 22) but none were found on filaments. The antheridium is globular or slightly elongated (Figs. 25, 26, 33, 34). It develops a wall consisting of a basal cell, a ring cell and an undivided cap cell (Figs. 23-26, 28-30, 35) surrounding a mass of spermatogenous cells which usually touch the base of the antheridium (Figs. 24-26, 29, 30) but not in all cases (Figs. 33, 34). The sperms are discharged through a pore (Figs. 27, 36). On the posterior part of old prothalli the antheridia may exhibit irregularities. Some of the antheridia are very large (Figs. 36,



Figs. 23-38 — Antheridium. Figs. 23-26. Stages in development, surface view. Fig. 27. Discharge of sperms through pore. Figs. 28-30. L.S. stages in development. Fig. 31, Young stage of large antheridium with divided basal cell, surface view. Fig. 32. L.S. antheridium with divided basal cell. Figs. 33, 34. External view. Fig. 35. Top view. Fig. 36. Discharge of sperms, large antheridium. Fig. 37. L.S. antheridium with divided basal cell. Fig. 38. Large antheridium with divided basal cell. All, × 320.



FIGS. 39-53 — Archegonium. Figs. 39-47. L.S. of stages in development. Fig. 48. L.S. opening of neck. Fig. 49. Mature archegonium. Fig. 50. Open neck seen from above. Fig. 51. L.S. open neck. Fig. 52. Side view of open neck. Fig. 53. L.S. thallus with stages of archegonia. Figs. 39-49. × 320. Figs. 50-53. × 200.

38), but since the sperms in them are discharged and swim, it is presumed that the antheridia are functional. They sometimes show a divided basal cell (Figs. 31, 37, 38), but antheridia of the usual size may also show this (Fig. 32). Davie (1951) figures and describes a divided basal cell in *Cyclosorus parasitica* (L.) Farwell. The fact that our cultures were made from spores of greenhouse plants might explain the occurrence of these irregularities.

Archegonium

The archegonium develops in the customary way (Figs. 39-47) from an initial cell 4 or 5 segments behind the apical initials (Fig. 53). The nucleus of the basal cell is somewhat larger than that observed in other species (Figs. 43-45), but this may be because it divides relatively late (Figs. 46, 47). The neck canal nucleus begins division slightly before that of the central cell (Fig. 46). The neck of the mature archegonium is 4-6 cells long (Figs. 48, 49) and bends away from the notch (Figs. 21, 48, 49, 51, 53). There are often conspicuous vacuoles at the tip of the neck canal cell (Fig. 47), and the ventral canal cell degenerates early (Figs. 47-49). The lower cells of the neck undergo secondary divisions (Figs. 47-49, 51), and these sometimes take place before the elongation of the neck cells which indicates maturity (Fig. 47). Archegonia are occasionally found on the dorsal surface. This may be a result of overlapping in the cultures. Although in the developing archegonium

the usual walls appear, they may do so in a plane slightly different from that expected, as in the lower wall of the neck initials seen in Fig. 39 at the right. These departures from the usual seem to be well within the functional limits of physiological variation, because the necks of the archegonia open (Figs. 48, 50-52) and sporophytes develop.

Discussion

Christensen (1938) placed *Didymochlaena* in his Dryopteridoideae among the polystichoid derivatives. Holttum (1949) put the genus in his sub-family Dryopteridoideae of his Dennstaedtiaceae — a " peculiar pantropic genus " — and said that its relation to the other genera is neither near nor precisely definable. Copeland (1947) placed *Didymochlaena* in his Aspidiaceae, and stated that it has " significant characters in common with *Dryopteris* and with *Polystichum* ", and that it is reasonable to suppose that *Polystichum* is its nearest relative; that it is an old genus which may be related to *Polystichum* without being a descendant. Ching (1940) considered *Didymochlaena* an isolated genus and established the family Didymochlaenaceae for the monotypic genus, saying that it is a peculiar family of uncertain position, although generally associated with the Aspidioid ferns. It should be noted that Ching described the spores of *Didymochlaena* as " large, tetrahedral without perispores, with 3 radiating deep, broad grooves ", and gives the spore character among the reasons for establishing the new family. Copeland (1947) described the spores of *Didymochlaena* as " bilateral, oblong to roundish, angular or irregularly tuberculate by shrinkage of epispore, dark ". Our material corresponds to the characterization of spores given by Copeland. Dickason (1946) recognized the family, although he did not place it with the Aspidioid ferns but with the Davalliaceae, Dennstaedtiaceae and others.

This investigation was undertaken to see if the gametophyte gave any indication of the position or relationship of *Didymochlaena*. We have found nothing in the development or structure to justify the establishment of a family, and very little to suggest relationships. In general it conforms to the type of gametophyte most common among the higher ferns, producing a cordate or reniform thallus with the usual type of sex organs. Its early stages are suggestive of the more primitive among the higher ferns in the following points: chloroplasts are abundant in young rhizoids, the plate stage arises early, long filaments are unusual, branching filaments are rare, ameristic male prothalli when formed are plate-like rather than filamentous, and the thallus has a low degree of plasticity. The extent to which marginal rhizoids are developed is unusual in higher ferns, so far as known, but they are characteristic of the ribbon-like gametophyte of *Elaphoglossum* sp. (Stokey, 1951) in which their development is much more extensive and persistent.

The mature gametophyte of *Didymochlaena* is like most of those described for Copeland's Aspidiaceae with the exception of the ribbon-like thallus of *Elaphoglossum*. It differs from the majority of that family so far described in having a naked thallus — a thallus devoid of hairs. The family in general, particularly the genera *Dryopteris* and *Polystichum*, are characterized by hairs which are present on the thallus in greater or less abundance. However, there is a scattered minority in this family which has naked gametophytes. Lagerberg (1908) described the prothalli of *Athyrium alpestre* (Hoppe) Rylands, and *A. filix femina* (L.) Roth as naked, and Faegri (1934) that of *A. crenatum* as naked, but Freer (1926) reported hairs on *A. pycnocarpon* (Spreng.) Tidstr. [*Asplenium angustifolium* Mich]. Lagerberg described the prothallus of *Matteuccia struthiopteris* (L.) Todaro as naked. The prothallus of *Onoclea sensibilis* L., however, has an abundance of hairs. In our cultures we have found that the gametophytes of *Bolbitis cladorrhizans* (Spr.) Ching and that of *Hemidictyum marginatum* (L.) Presl are naked. More information is needed about the presence and type of hairs in the various genera. In general, as families of ferns are considered, it appears that the naked thallus is an earlier type than that with hairs.

The anomalies of the antheridium are unusual in that they affect the basal cell and not the cap cell. It is probable that their significance is slight, as may be the case, also, with the irregularities in the cap cell which are so widely distributed among genera and families of the higher ferns.

Copeland said of *Didymochlaena* that "it must be an old genus". The habit of development of the gametophyte, the primitive character of the rhizoids and the lack of hairs on the thallus give support to that statement.

Summary

The gametophyte of *Didymochlaena sinuata* arises as a short filament soon followed by a plate which becomes cordate or reniform at maturity. Chloroplasts may be abundant in the rhizoids even in the cordate stage of the thallus; marginal rhizoids are abundant until near maturity, which the gametophyte attains in 10-11 weeks; only ventral rhizoids are formed on older thalli. The antheridia are of the type characteristic of higher ferns, but a wall forms occasionally in the basal cell. No hairs are formed at any time.

Part of this investigation was carried out by the senior author at the Marine Biological Laboratory, Woods Hole, Mass.

The junior author wishes to thank the Biology Department of Amherst College for materials and for many courtesies.

Literature Cited

Ching, R. C. 1940. On the natural classification of the family "Polypodiaceae". Sunyatsenia **5**: 201-268.

Christensen, C. 1906. "Index Filicum." Copenhagen.

— 1938. Chap. 20 in Verdoorn's "Manual of Pteridology." Waltham, Mass.

Copeland, E. B. 1947. "Genera Filicum." Waltham, Mass.

Davie, J. H. 1951. The development of the antheridium in the Polypodiaceae. Am. Jr. Bot. **38**: 621-628.

Dickason, F. G. 1946. A phylogenetic study of the ferns of Burma. Ohio J. Sci. **46**: 73-108.

Döpp, W. 1927. Untersuchungen über die Entwicklung von Prothallien einheimischer Polypodiaceen. Pflanzenforschung **8**: 1-58.

Faegri, K. 1934. Zur Kenntnis der Prothallien von *Athyrium crenatum* und *Asplenium marinum*. Bergens Museums Årbok. Nr. 12.

Freer, R. S. 1926. Notes on the development of the gametophyte and embryo of *Asplenium angustifolium*. Ohio J. Sci. **26**: 147-168.

Holttum, R. E. 1949. Classification of ferns. Biol. Rev. (Cambridge) **24**: 267-296.

Lagerberg, T. 1908. Morphologische-biologische Bemerkungen über die Gametophyten einiger Schwedischer Farne. Svensk Bot. Tidskr. **2**: 229-276.

Stokey, A. G. 1951. The contribution of the gametophyte to the classification of the homosporous ferns. Phytomorphology **1**: 39-58.

THE COAL-AGE FLORA OF KANSAS — V. A FOSSIL CONIFEROPHYTE WOOD

R. W. BAXTER & EMILY L. HARTMAN
University of Kansas, Lawrence, Kansas, U.S.A.

In the summer of 1952, Dr. Frank Peabody of the University of Kansas, while searching for fossil reptile skeletons, conducted extensive explorations in the area of Garnett, Kansas. This area has for long been of great palaeontological interest in that, particularly regarding its fossil flora, it seems to show a Permian facies in Pennsylvanian strata. The investigations of Moore, Elias and Newell (1936) established the correct stratigraphy as being in the Lansing group in the middle part of the Kansas Pennsylvanian, and provided one of the first detailed lists of the compression genera constituting the known flora of which the dominant components appear to be *Walchia* and a new genus established by Elias which he called *Dichophyllum moorei*.

The intermingling of common Permian genera such as *Taeniopteris* and *Walchia* with numerous plants of essentially Pennsylvanian aspect in strata definitely proven to be Pennsylvanian has given rise to a number of intriguing ecological theories. Among the most interesting of these is that the environment was a relatively xeric one surrounding an inland lagoon or embayment in the waters and sediments of which the present fossil record was preserved. While geologically the flora was contemporaneous with that forming much of the eastern Kansas coal beds, the make-up was strikingly different. The strong dominance of *Walchia* suggests an almost coniferous homogeneous forest in which the usual Upper Carboniferous plants, *Lepidodendron*, *Calamites*, etc., were present but in a very minor role. Peabody's (1952) investigations of *Petrolacosaurus Kansensis* seems to show that this primitive Pennsylvanian reptile was an agile terrestrial form which probably lived in the *Walchia-Dichophyllum* forest.

It was accordingly a matter of considerable interest when the large collections made by Dr. Peabody resulted in the discovery of a fragment of charcoalized (fusain) wood embedded in the same shale in which are found the numerous plant compressions. The wood specimen was not in organic connection with the carbonaceous material of any of the compressions, so definite correlation with known genera has not been possible. However, the specimen exhibits certain anatomical characters which so limit the groups to which it could be assigned that through a process of elimination we find that we can postulate an identification with one of the dominant genera, *Walchia* and *Dichophyllum*.

Elias (Moore, Elias & Newell, 1936) compiled the following list which, while representing preliminary identifications and including a number of doubtful designations and unvalidated new species, still constitutes the most complete record available of the Garnett fossil flora:

" *Palaeophycus bullatus* Elias, n. sp.
Palaeophycus n. sp.
Annularia gallioides Lindley & Hutton (= *A. spicata* Gutbier)
Sphenopteris (*Aneimioides*) *zeilleri* Elias, n. sp. (= *S. polyphylla* Zeiller, 1886, 1888, not Lindley & Hutton)
Sphenopteris (*Discopteris*) cf. *goldenbergi* Andrae
Sphenopteris cf. *minutisecta* Fontain & White
Pecopteris cf. *abbreviata* Brongniart
Neuropteris fimbriata ? Lesquereux, pinnule with attached seed-like body

Neuropteris (*Mixoneura*) *whitei* Elias, n. sp. (belongs to the group of *N. impar* and *N. acuminata*)
Odontopteris (*Mixoneura*) *sternbergii* mut. *a* Elias, n. mut. (= pars. *O. obtusa* Weiss, 1869-72, Pl. III, Fig. 5 only)
Odontopteris n. sp., aff. *Neuropteris callosa* Lesquereux and *N. odontopteroides* Fontain & White
Callipteridium grandifolium Fontain & White
Alethopteris kansasensis Elias, n. sp. (belongs to *Alethopteris lonchitica* group)
Alethopteris sp. cf. *massillonis* Lesquereux
Desmopteris n. sp. A
Desmopteris n. sp. B
Taeniopteris coriacea Goeppert
Taeniopteris cf. *newberriana* Fontain & White (= *T. multinervis* Weiss)
Taeniopteris cf. *angelico* D. White
Taeniopteris n. sp.
Pteridospermostrobus stopesi Elias, n. sp.
Lepidophyllum? cf. *tumitum* Lesquereux
Cordaites n. sp., aff. *borassifolius*
Cordaites n. sp., aff. *clerci* Zalessky
Samaropsis n. sp. A
Samaropsis americana Elias, n. sp.
Samaropsis n. sp. B
Samaropsis sp.
Dicranophyllum? *garnettensis* Elias, n. sp.
Walchia piniformis (Schlotheim) *sensu stricto*
Walchia filiciformis Sternberg
Walchia cf. *pinnata* Gutbier cf. *Walchia* (*Trichomanites*) *frondosa* (Goeppert)
Voltzia foliosa Elias, n. sp. cf. *liebeana* Geinitz
Ullmania? sp.
? *Lecrosia* n. sp. (or n. gen., n. sp.)
Dichophyllum moorei Elias, n. gen., n. sp.
Dichophyllum sp."

As can be seen from a perusal of the above compression genera, the major vascular groups represented include [according to Arnold's (1948) classification], the Pteridospermae (*Alethopteris*, *Neuropteris*, etc.), the Cycadeoidales, or Cycadales (*Taeniopteris*), the Sphenopsida (*Annularia*), the Lycopsida (*Lepidophyllum*), the Cordaitales (*Cordaites*, *Walchia*, etc.) and possibly the Ginkgoales (*Dicophyllum*). Elias's assignment of certain specimens to *Dicranophyllum* was admitted by himself to be questionable. In addition, the taxonomic position of the genus seems so doubtful that it serves little purpose here to theorize relationships with other organ genera.

The essential characters of the wood are well known for all of the above major taxonomic groups. All of them, except the Cordaitales and Ginkgoales, have a manoxylic type wood with large thin-walled tracheids and abundant parenchyma or broad rays. The Cordaitales and Ginkgoales alone (of the groups here represented) have a pycnoxylic-type wood with compact small thick-walled tracheids and narrow rays which Arnold (1948) makes a basic character of his class, Coniferophyta.

Preliminary investigations have shown this latter type to be that of our fossil wood. The problem thus presented has accordingly been to determine in just what ways the present specimen differs from the known *Cordaites* and *Mesoxylon* wood, with the assumption that the existence of distinguishing characters would, through elimination of all other possibilities, indicate an assignment to the *Walchia* complex of organ genera or the Ginkgoales.

General Description — The specimen was preserved in a non-mineralized charcoalized condition which Hambleton (1953) describes as fusain or mineral charcoal. This particular type of preservation is characterized by having a fibrous, dull or opaque appearance and being composed of maceral fusinite. It consists of a fragment of secondary xylem 4·5 × 1·5 cm. in diameter and 3·0 cm. in length (Fig. 1). The wood is quite soft and pieces were easily cut out of it with a razor blade, the only disadvantage being the tendency for smaller fragments to crumble into a fine dust. The larger pieces cut from the specimen were treated with a dilute solution of hydrofluoric acid for 4-6 hours and then quickly run up to absolute alcohol, into xylol and embedded in paraffin. Thus prepared, transverse, radial and tangential sections were cut at 8-15 microns on a rotary microtome with the subsequent steps leading to the finished slides being the same as those in normal microtechnique, except that here

FIGS. 1-3 — Fig. 1. Fragment of charcoalized wood embedded in shale. × 1·3. Fig. 2. Transverse section made from specimen shown in Fig. 1. Indication of an annual ring can be seen near top left portion of section. × 22. Fig. 3. Radial section of *Dicranophyllum* wood showing occasional small rays evident as horizontal bands. × 42.

no staining was necessary due to the cell walls already being black or dark brown in colour. Although extensive anatomical detail was rendered impossible by the inadequate preservation, the authors found that cell measurements and related observations of cellular structure could be obtained.

In transverse section (Fig. 2) the wood shows the general characters associated with a coniferous type wood. The tracheids tend to be of uniformly small size, averaging 25 × 18 μ with their radial diameter being the larger. Xylem parenchyma seems to be lacking while, the sparseness of the small, uniseriate rays gives the wood a very compact homogeneous appearance.

Reliable evidence as to the presence or absence of annual rings as indicators of climatic conditions is lacking. Due to the friability of the fusain material it proved impossible to section pieces larger than 5-6 mm. in diameter so that any one piece was possibly less than the width of a single annual ring, if such were present. One specimen, Fig. 2, seemed to show a definite line of demarcation between relatively large thin-walled tracheids and much smaller tracheids with thicker walls, which might represent a fragment of the juxtaposition of summer and spring wood, although, as stated above, the section was too small to provide a real basis for evaluation of this character.

In radial section the rays can be observed as horizontal bands 1-4 cells in height (Fig. 3). The tracheids show reasonably well-preserved bordered pits on their radial walls. The pits average 9·75 μ in diameter with a circular border and slit-like apertures at approximately a 45° angle from the horizontal, opposing apertures being arranged at a 90° angle to each other so that they present an X-shaped pattern (Fig. 6). The pits are characteristically in uniseriate rows although occasionally a cell may show a double row with the pits being opposite one another rather than alternate.

The tangential walls generally seemed to be poorly preserved and few definite observations could be made. A few sections of intact tangential tracheid wall showed no pitting to be present.

With the above general description of this Mid-Pennsylvanian wood, we may now proceed to reapproach the problem of identification. As stated earlier, the known compression flora from the strata includes representatives of the Pteridospermae, the Cycadeoidales or Cycadales (*Taeniopteris* leaves being known to occur in both groups), the Sphenopsida, the Lycopsida, the Cordaitales and the Ginkgoales. Of these groups the only ones showing similar wood characters to our present specimen are the Cordaitales and Ginkgoales. Accordingly, we are either dealing with a fragment of *Cordaites* or *Walchia* (both of which are present in the compression genera) or we may assume that the wood is of a plant related to these woods but distinct enough from them taxonomically to be distinguished by variations in wood anatomy.

The details of wood anatomy in *Cordaites* and *Mesoxylon* (the two not differing in the character of the secondary xylem) are well known. Little, however, is known of the internal structure of *Walchia*. Elias (1948) gives a short account from observations of stem material obtained from an organically preserved compression of a *Walchia* branch. He reports that the wood is characterized by numerous biseriate rays with the tracheid pitting consisting of double rows of alternate hexagonal pits. On the basis of these characters our present coniferophyte wood obviously cannot be assigned to *Walchia* so that only *Cordaites* remains to be eliminated in order to leave the Ginkgoales as the sole possibility.

Table 1 below outlines a number of comparative wood characters for *Cordaites* (from Mid-Pennsylvanian coal balls), the Garnett specimen and wood of the living *Ginkgo biloba* and *Pinus* sp.

It can be seen that in tracheid diameter, ray size and pit dimensions our unknown wood is quite distinct from *Cordaites* and, in fact, compares much more closely to the living *Ginkgo*.

Discussion

The striking similarity of the Garnett specimens to the wood of the living *Ginkgo biloba* seems to be of special interest. The

TABLE 1

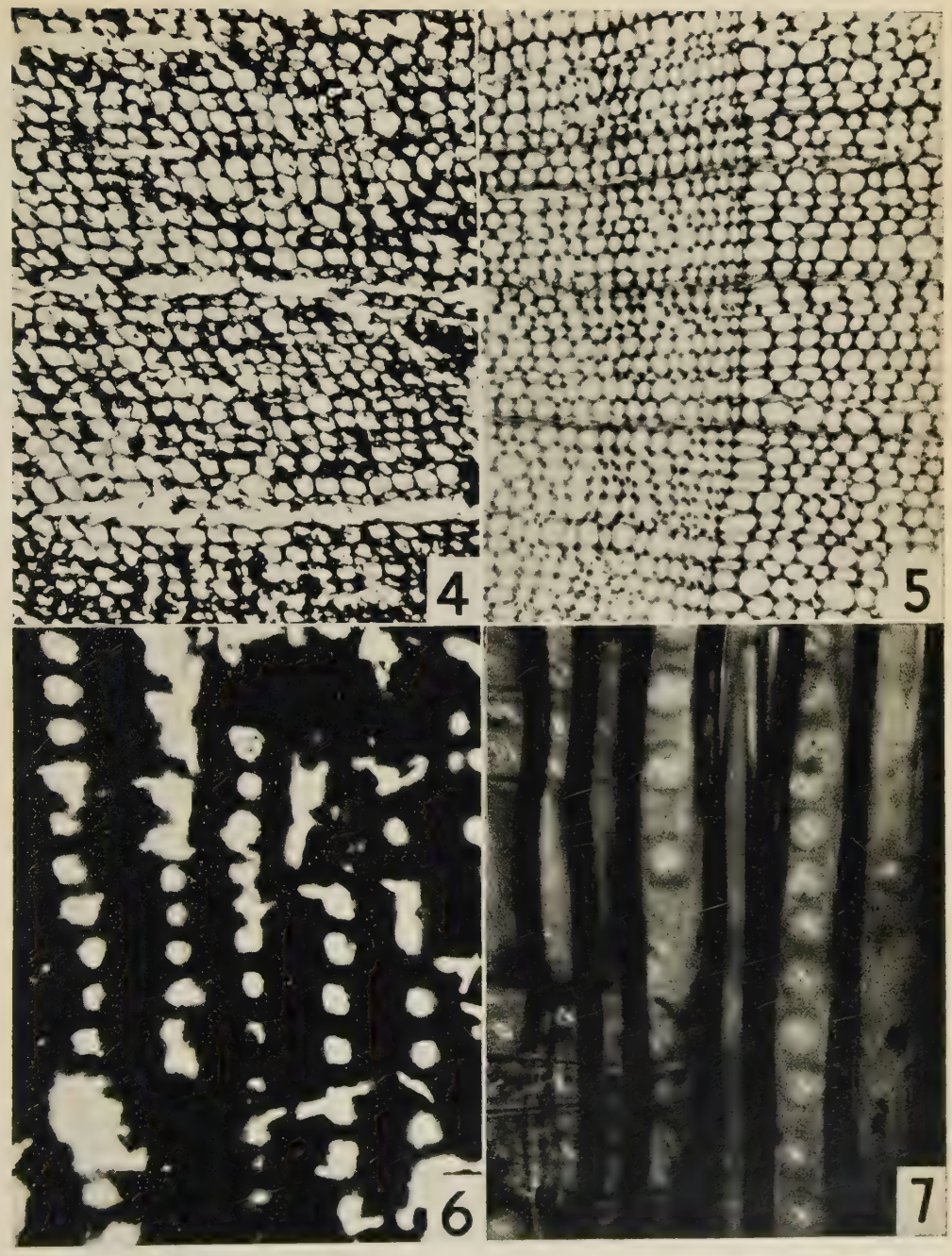
Plant	Average diameter of tracheids	Wood rays	Average pit diameter	Pit characters	Annual rings
Garnett specimen (Dichophyllum wood ?)	25 × 18 μ Radial diameter the larger	Uniseriate 1 to 5 cells high	9·75 μ	Borders — round Apertures—diagonal slits Predominantly uniseriate but occasionally in double rows	Weakly defined or absent
Ginkgo biloba	23 μ × 15 μ Radial diameter the larger	Uniseriate 1 to 4 cells high	11 μ	Borders —round Apertures—oval to diagonal slits Predominantly uniseriate but sometimes in double rows	Weakly defined
Cordaites and *Mesoxylon*	47 μ × 30 μ Tangential diameter the larger	Uniseriate 2 to 5 cells high	15 μ	Borders—rounded hexagonal Appertures—diagonal slits Double rows arranged alternately	Absent
Pinus sp.	52 × 42 μ in spring wood: Tangential diameter larger	Uniseriate 3 to 10 cells high	27 μ	Borders — round Apertures — round Predominantly uniseriate but occasionally in double rows	Well defined

fossil record of the Ginkgoales is based almost entirely on leaf imprints and is generally admitted to extend as far back as late Permian, although leaves which are definitely assignable to the genus *Ginkgo* probably date no earlier than late Triassic or early Jurassic. Sellard (1908) has reported *Baiera* sp. from the Wreford limestone in Dickinson County, Kansas, which is generally identified as of Permain age. However, since he seems to have been doubtful of the assignment to the genus and gives little description and no illustrations, the record may be viewed with some scepticism.

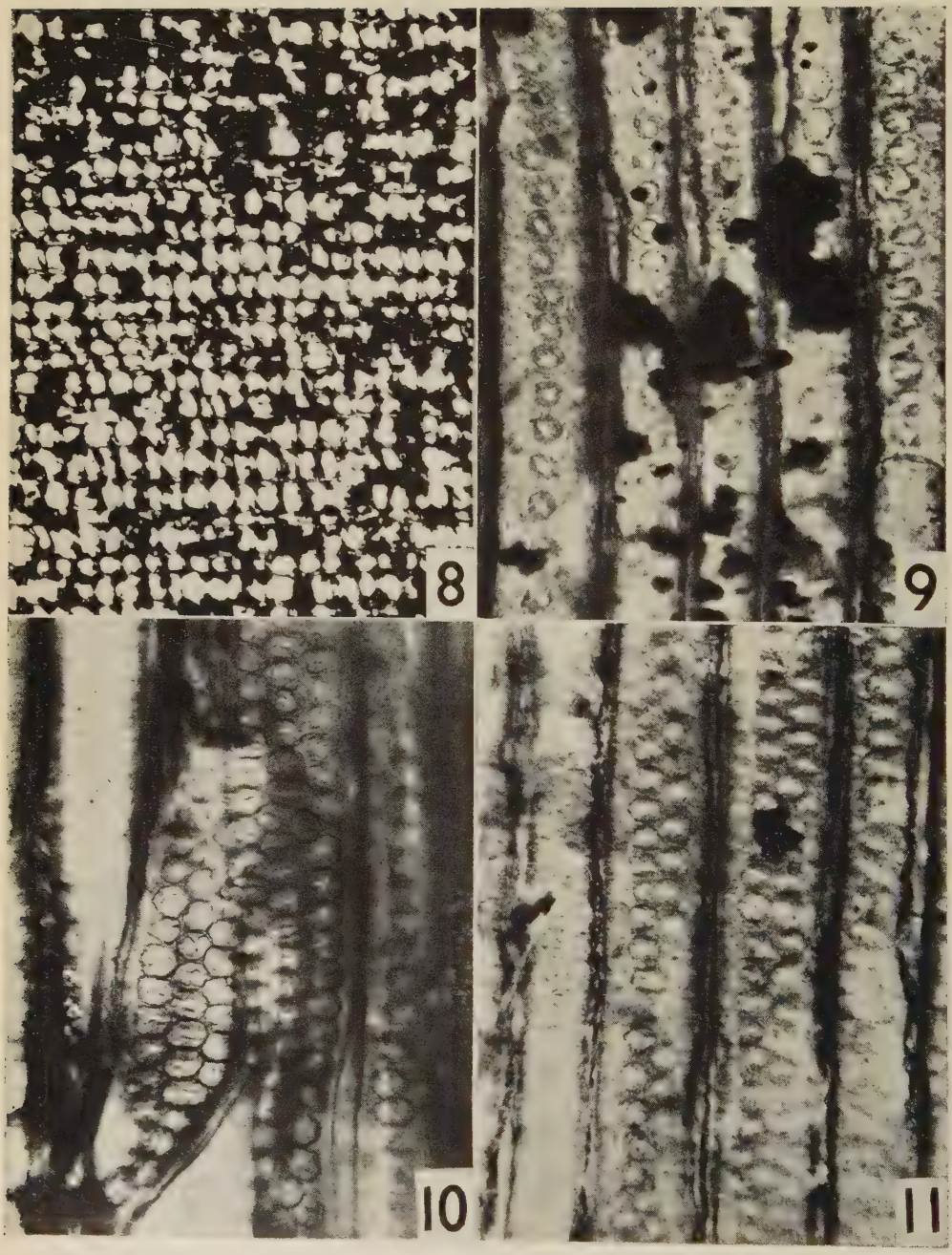
Dichophyllum moorei Elias (Fig. 12), which, according to Peabody, constituted approximately 30 per cent of the plant compressions uncovered (*Walchia* constituting about 60 per cent with the remaining 10 per cent consisting of the fourteen other genera listed), has been postulated by Andrews (1941) as a possible *Ginkgo* progenitor. As shown in Fig. 12, the leaf (or flattened telomes) present a fan-shaped pattern somewhat comparable to that of the *Ginkgo* leaf. That the fragmentary "leaf" compressions were part of a large woody plant capable of producing abundant secondary xylem is shown by the size of some of the specimens, one branch having been recovered with a length of over 2 ft. and a basal stem diameter of approximately ½ in.

Accordingly, it is felt that on the basis of the present evidence, the most likely assignment of the fossil wood specimen herein described is with the Ginkgoales and that, in that sense, it constitutes an important bit of additional evidence for Andrews's (1941) theory that *Dichophyllum moorei* may represent a primitive progenitor of the genus *Ginkgo*.

As shown in Figs. 4-7, the tracheid size and shape are practically identical for the fossil wood and the wood of *Ginkgo biloba*. In both, the annual rings are inconspicuous, the distinction between spring and summer wood being relatively slight which, as Seward (1919) points, out is a feature distinguishing *Ginkgo* wood from that of the conifers. The pitting in both is almost completely uniseriate with the diameter of the pit borders being nearly



FIGS. 4-7 — Fig. 4. Enlargement of transverse section of *Dichophyllum* wood. × 180. Fig. 5. Transverse section of *Ginkgo biloba* wood. × 180. Note inconspicuous merging of annual rings. Both transverse sections are shown at identical magnifications. Fig. 6. Radial section of *Dicho phyllum* wood. × 600. Fig. 7. Radial section of *Ginkgo biloba* wood. × 600. Both radial sections are shown at same magnification.



FIGS. 8-11 — Fig. 8. Transverse section of *Cordaites* sp. wood. Note greater tangential diameter of tracheids. × 180. Figs. 9, 11. Radial sections of *Cordaites* sp. wood from coal ball material. × 600. Fig. 10. Radial section of *Cordaites* sp. wood from silicified specimen. × 600.

FIG. 12 — *Dichophyllum moorei*. Neotype specimen. × 1.

identical in both, and consistently smaller than the pit borders in both *Cordaites* and pine.

The greater radial diameter of the tracheids constitutes still another common character for our fossil wood and that of

Ginkgo in contrast to the situation in *Cordaites* wood where the tangential diameter is commonly the greater (Fig. 8).

Different types of radial pitting found in *Cordaites* wood are illustrated in Figs. 9-11. Figs. 9 and 11 show uniseriate and biseriate pitting in *Cordaites* sp. from stems found in the coal balls of West Mineral, Kansas (Baxter, 1951). The features serving to distinguish our present fossil wood specimen from *Cordaites* are the larger pit borders in the latter, the tendency of the pits to have an alternate arrangement when in double rows rather than opposite, as is the case in the Garnett fossil wood, and the much larger tracheid diameter. The pitting shown in Fig. 10 is from a silicified specimen of *Cordaites* from the Pennsylvanian of Indiana and serves to illustrate the closely grouped hexagonal pits which are usually described as being characteristic of *Cordaites*, but which, in fact, very few of the American coal ball *Cordaites* stems have.

In view of the above features showing the similarity of the Garnett fossil wood specimen to the Ginkgoales and important differences when compared to the Cordaitales, the only member of the known compression flora with which it seems logical to associate it, is *Dichophyllum*.

Inasmuch as the above viewpoint adds greatly to the significance of the compression genus, *Dichophyllum*, it is felt that its taxonomic position should be clarified. Elias (Moore, Elias & Newell, 1936) listed *Dichophyllum moorei* as a new genus and species and illustrated it, but did not give any diagnosis or designate any type specimen. Andrews (1941) gave the first general description of the genus and illustrated it with six photographic plates as well as an artist's reconstruction. However, in accepting Elias as the author of the genus, he also neglected to correct the original oversight in the omission of a generic diagnosis and designation of type specimens.

Accordingly, the following descriptive diagnosis is offered in line with Art. 58 of the International Code of Botanical Nomenclature in order to definitely validate the genus:

Dichophyllum moorei Elias *ex* Baxter & Hartman. A flattened branch or dissected leaf. Major subdivisions essentially dichotomous but with a tendency towards sympodial development. Dichotomies sometimes so closely grouped as to resemble palmate divisions. Ultimate telomes or pinnules dichotomous in closely grouped pairs 4-5 cm. long and 1·2 mm. wide.

Horizon: Victory Junction member of the Stanton Limestone, Missouri Series. Upper Carboniferous.

Neotype: Specimen Kan.-1/A 8356 University of Kansas Collection of Fossil Plants. Fig. 12.

Summary

A seemingly xerophytic upper Pennsylvanian flora first described by Elias (Moore, etc., 1936) is discussed. The known compression flora consisting of a high percentage of *Walchia* and other common Permian genera is listed.

A piece of charcoalized (fusain) wood found embedded in the above plant fossil bearing shale is described and comparative characters listed to show that among the most likely possible taxonomic assignments the closest resemblance is the Ginkgoales.

The genus *Dichophyllum moorei* Elias *ex* Baxter & Hartman is redescribed and a neotype specimen illustrated. Evidence is offered to show that the fossil wood may be considered to be that of *Dichophyllum* and that in its *Ginkgo* anatomy it provides additional weight to Andrews's (1941) theory of *Dichophyllum* being a Pennsylvanian progenitor of *Ginkgo*.

Literature Cited

Andrews, H. N. 1941. *Dichophyllum moorei* and certain associated seeds. Ann. Mo. Bot. Gar. **28**: 375-384.

Arnold, C. A. 1948. Classification of gymnosperms from the viewpoint of palaeobotany. Bot. Gaz. **110**: 2-12.

Baxter, R. W. 1951. Coal balls — new discoveries in plant petrifactions from Kansas Coal Beds. Trans. Kansas Acad. Sci. **54**: 526-535.
Elias, M. K. 1948. *Walchia*: Anatomy of branch and leaf. Geol. Survey, America Bull. **59**: 1319-1320.
Hambleton, W. W. 1953. Petrographic study of south-eastern Kansas coals. Geol. Surv. Kansas Bull. **102** (1): 1-76.
Moore, R. C., Elias, M. K. & Newell, N. D. 1936. A " Permian " flora from the Pennsylvanian rocks of Kansas. J. Geology **44**: 1-31.
Peabody, F. E. 1952. *Petrolacosaurus Kansensis* Lane, A Pennsylvanian reptile from Kansas. Univ. Kansas Palaeont. Contributions Vertebrata, Article 1, 1-41.
Sellards, E. H. 1908. Fossil plants of the Upper Palaeozoic of Kansas. The Permian flora of Kansas. Univ. Geol. Surv. Kansas **9**: 386-467.
Seward, A. C. 1919. " Fossil Plants." Vol. IV.

EMBRYOLOGIE DE L'*ERINUS ALPINUS* L. (SCROFULARIACÉES). LES RELATIONS ENTRE LES GENRES *ERINUS* L. ET *DIGITALIS* L.*

PIERRE CRÉTÉ

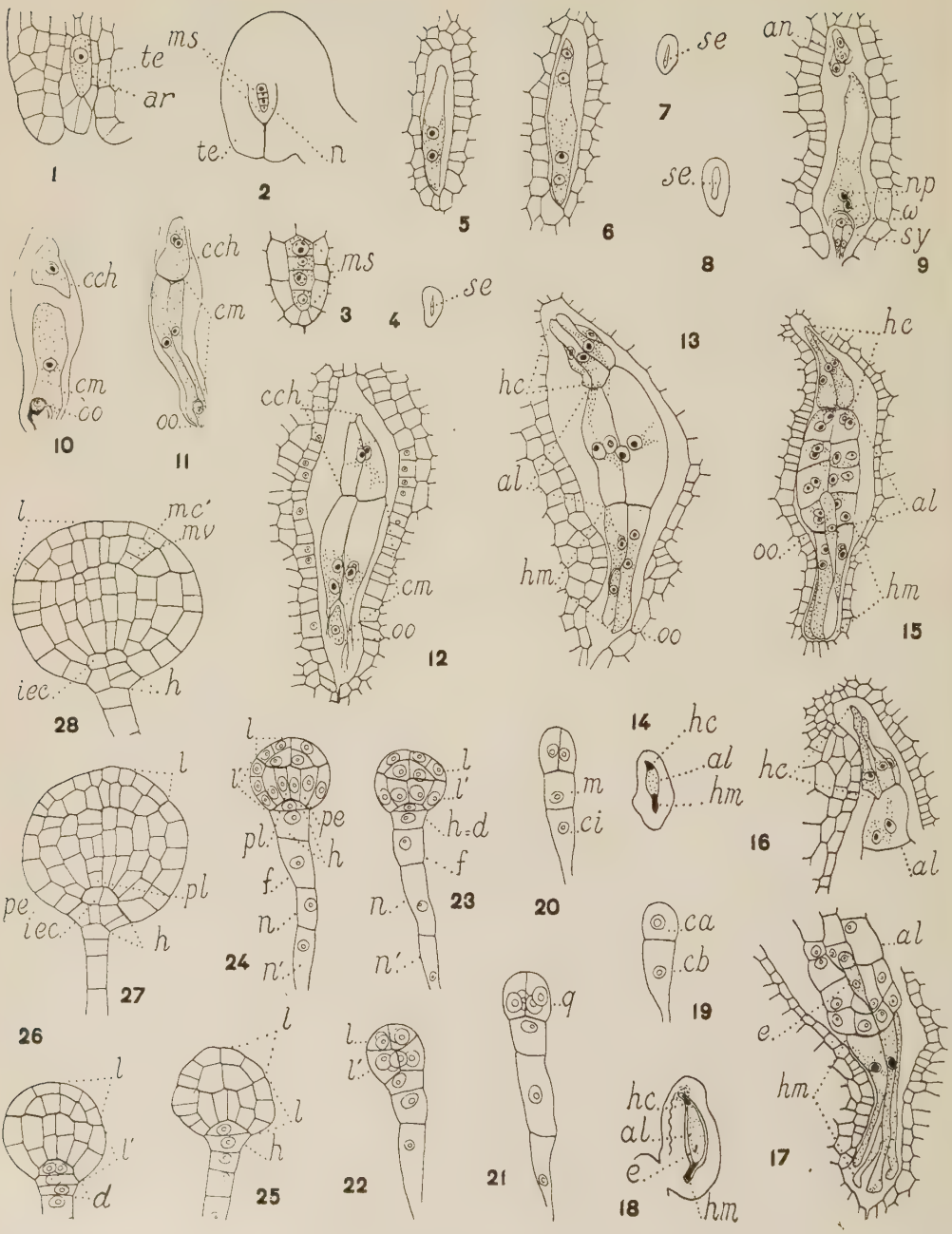
Faculté de Pharmacie, Université de Paris, France

Dans la classification d'Engler et Prantl, les *Erinus* avoisinent immédiatement les *Digitalis*; comme eux, ils font partie, dans la sous-famille des Rhinanthoïdées, de la tribu des Digitalées; dans celles de Baillon, d'une part (1888), et de Bentham et Hooker, d' autre part, ils font partie côte à côte de la série ou tribu des Digitalées et de la sous-série ou sous-tribu des Eudigitalées. Ayant tout récemment mis au point l'étude du développement de l'albumen et de l'embryon chez le *Digitalis purpurea* L. (Crété, 1953), j'ai cru utile de vérifier si des caractères embryologiques voisins confirmaient les relations étroites que la morphologie externe permet d'établir entre les Digitales et les *Erinus*. L'*Erinus alpinus* L., sujet de cette étude, a été récolté dans le Jardin Botanique de la Faculté de Pharmacie de Paris. Il est voisin des *Digitalis* parce que c'est une plante vivace, à feuilles caulinaires alternes, courtement pétiolées, à fleurs purpurines disposées en grappes terminales; la corolle est tubuleuse, le stigmate est bilobé. Mais les *Digitalis* sont des plantes robustes; leurs fleurs, penchées, sont réunies en une grappe unilatérale et elles ont une longue corolle à tube ventru, à limbe oblique présentant quatre ou cinq lobes courts et très inégaux; les anthères sont à deux loges. Les *Erinus* sont au contraire des plantes gazonnantes; les fleurs, dressées, sont disposées en des grappes affectant la forme de corymbes; les corolles sont petites, à tube grêle, à limbe presque plan et présentent cinq lobes échancrés, subégaux; les anthères sont à une loge seulement.

Origine et structure du sac embryonnaire

L'archéspore unicellulaire et sous-épidermique (Fig. 1) donne naissance à des macrospores disposées en une tétrade

*Les résultats de ce travail ont été présentés au 8. Congrès international de Botanique à Paris, mais il n'en a paru qu'un court résumé sous le titre: " Le gamétophyte femelle, l'albumen et l'embryon chez l'*Erinus alpinus* L. (Scrofulariacées) ".


te
ar
1
ms
te
n
2
5
6
se
7
se
8
an
np
ω
sy
9
cch
cm
co
10
cch
cm
oo
11
ms
3
4
se
13
hc
al
hm
oo
hc
al
oo
hm
15
cch
cm
oo
12
l
mc'
mv
iec
h
28
l
l'
pe
pl
f
n
n'
24
l
l'
h
d
f
n
n'
23
m
ci
20
14
hc
al
hm
hc
al
16
l
pe
pl
iec
h
27
26
ca
cb
19
q
21
l
l'
d
l
l
h
25
l
l'
22
hc
al
e
hm
18
al
e
hm
17

FIGS. 1-28.

linéaire. C'est la macrospore interne, la macrospore chalazienne, qui est la cellule-mère du sac embryonnaire; cependant, des cellules intermédiaires de la tétrade peuvent atteindre, à un moment, des dimensions à peine inférieures à celles de la spore fonctionnelle (Figs. 2 et 3). Le sac embryonnaire adulte, obtenu après trois séries de divisions nucléaires (Figs. 4 à 7) renferme une oosphère, deux synergides, trois antipodes et deux noyaux polaires dont la réunion en un noyau secondaire est un fait accompli avant la fécondation (Figs. 8 et 9).

Développement de l'albumen

La première division du noyau d'albumen s'accompagne de la formation d'une paroi transversale (Fig. 10). Puis dans chacun des deux étages chalazien et micropylaire, *cch* et *cm*, il se différencie deux cloisons longitudinales se coupant à angles droits (Figs. 11 à 14). Les quatre cellules de l'étage chalazien prennent rapidement les caractères d'un haustorium: elles ne se divisent plus et présentent un cytoplasme très dense; cependant les diamètres nucléaires restent presque toujours inférieurs à ceux des noyaux de l'albumen proprement dit (Figs. 13, 15 et 16). Dans l'étage micropylaire, des cloisonnements transversaux interviennent à un même niveau de chacune des cellules, isolant deux nouvelles tétrades dont une est à l'origine de l'albumen proprement dit et l'autre d'une seconde formation haustoriale (Fig. 13). C'est ainsi que les quatre initiales de l'albumen proprement dit se divisent transversalement, puis les cellules-filles une fois encore de la même façon (Fig. 15), après quoi les cloisonnements s'orientent diversement, permettant de la sorte l'épaississement de l'albumen. En ce qui concerne les initiales de l'haustorium micropylaire, elles s'agrandissent notablement, sans plus se diviser. A l'époque où l'embryon devient bicellulaire, les noyaux finissent par atteindre des dimensions relativement considérables, mais restent toujours à proximité de l'albumen proprement dit, au lieu de se déplacer, comme c'est la règle presque générale chez les Scrofulariacées, vers l'extrémité micropylaire des cellules (Fig. 17). Cet haustorium ne semble pas exercer une bien grande activité et ne provoque pas en particulier la lyse des cellules tégumentaires qui le limitent.

Développement de l'embryon

L'embryon appartient à la première période de la Classification embryogénique de Souèges. Il se rapporte au mégarché-type IV et, de façon plus précise, au sous-archétype que représente le *Capsella bursa-pastoris* (Figs. 19 à 28). Il est bicellulaire à la première génération (Fig. 19), quadricellulaire à la deuxième génération qui correspond au stade de la tétrade (Fig. 20); mais comme les cellules intermédiaire *m* et inférieure *ci* de la tétrade ne se cloisonnent jamais simultanément, le proembryon ne comprend plus que sept cellules à la troisième génération, qui correspond au stade des quadrants (Fig. 21). L'hypophyse tire son origine, non

---

←

FIGS. 1 à 28 — *Erinus alpinus* L. Développement de l'albumen et de l'embryon. De 1 à 9, origine et structure du sac embryonnaire; les schémas figurés en 2, 4, 7 et 8 correspondent respectivement aux détails figurés en 3, 5, 6 et 9. *te*, tégument ovulaire; *n*, nucelle; *ar*, archéspore; *ms*, tétrade linéaire des macrospores; *se*, sac embryonnaire; ω, oosphère; *sy*, synergides; *np*, noyaux polaires; *an*, antipodes. De 10 à 18, développement de l'albumen. En 14, schéma de la graine dont le sac embryonnaire est représenté en 13; en 18, schéma de la graine dont l'haustorium chalazien est figuré en 16 et l'haustorium micropylaire en 17. *cch* et *cm*, cellules chalazienne et micropylaire isolées lors de la première division de l'albumen; *hc*, haustorium chalazien et *hm*, haustorium micropylaire; *al*, albumen proprement dit; *oo*, oospore; *e*, embryon. De 19 à 28, les premiers stades du développement de l'embryon. *ca* et *cb*, cellule apicale et cellule basale du proembryon bicellulaire; *m*, cellule intermédiaire et *ci*, cellule inférieure de la tétrade; *q*, quadrants; *n* et *n'*, cellules-filles de *ci*; *d* et *f*, cellules-filles de *m*; *l*, octants supérieurs ou partie cotylée *sensu lato* H; *l'*, octants inférieurs ou partie hypocotylée; *de*, dermatogène; *pe*, périblème; *pl*, plérome; *h*, hypophyse; *ice*, initiales de l'écorce au sommet radiculaire; *mv* et *mc'*, initiales du méristème vasculaire et du méristème cortical des cotylédons. G.: 440 en 1, 3, 5, 6, 9 et de 19 à 28; 340 de 10 à 13; 275 en 15; 210 en 16 et 17; 200 en 2; 44 en 4, 7, 8, 14 et 18.

pas de la cellule intermédiaire *m* de la tétrade, mais de sa cellule-fille supérieure *d* (Fig. 23).

Conclusions

La macrosporogénèse nous est connue, chez les *Digitalis* grace aux travaux de Schmid (1906) sur les Scrofulariacées. Elle est identique à celle que je viens de décrire chez l'*Erinus alpinus*. L'embryogénèse est également comparable dans les deux genres, bien que le dermatogène se différencie simultanément chez les *Erinus*, successivement en général chez les *Digitalis*, dans les parties cotylée et hypocotylée. Cependant les caractères tirés du développement du sac embryonnaire et de l'embryon seraient insuffisants pour justifier un rapprochement des deux genres en question, tellement ils sont homogènes dans la famille tout entière. C'est la genèse de l'albumen, assez variable chez les Scrofulariacées, qui nous permettra de serrer plus étroitement le problème. On sait en effet que, sauf quelques exceptions, le tissu de réserve comporte des formations haustoriales, micropylaire et chalazienne. Presque toujours, l'haustorium micropylaire est quadricellulaire et les initiales de l'albumen proprement dit sont au nombre de quatre. Au contraire, l'haustorium chalazien présente une structure assez peu constante. Quadricellulaire chez les *Verbascum* et les *Celsia* (Håkansson, 1926), les *Teedia* (Crété, 1952) et les *Digitalis* (Crété, 1953) par exemple, il est tri- ou quadricellulaire chez les *Lvperia* (Crété, 1949), bicellulaire chez les *Chaenostoma* (Crété, 1948), unicellulaire et binucléé chez les *Linaria* (Persidsky, 1934) et les *Tetranema* (Crété, 1954) et unicellulaire et uninucléé chez les *Limosella* (Svensson, 1928) et les *Alonsoa* (Crété, 1950). L'état quadricellulaire du suçoir chalazien, chez l' *Erinus alpinus*, est un caractère à ajouter à ceux qui rapprochent par ailleurs les *Erinus* des *Digitalis*. On peut ajouter que, dans les deux cas, les formations haustoriales sont faiblement actives et présentent des noyaux de petites dimensions. Les caractères embryologiques viennent donc à l'appui du point de vue des systématiciens qui font des *Digitalis* et des *Erinus* deux groupements extrémement voisins.

Literature Cited

Baillon, H. 1888. " Histoire des plantes." Hachette édit., Paris.

Bentham, G. & Hooker, J. D. 1862-1883. " Genera Plantarum." London.

Crété, P. 1948. Recherches embryologiques chez les Scrofulariacées. Développement de l' albumen et de l'embryon chez le *Chaenostoma foetidum* (Jacq.) Benth. Bull. Soc. bot. France **95**: 142-146.

— 1949. Recherches embryologiques chez les Scrofulariacées. Développement de l'albumen chez le *Lyperia violacea* Benth. Bull. Soc. bot. France **96**: 186-188.

— 1950. Embryologie des Scrofulariacées. Développement de l'albumen chez l'*Alonsoa caulauliata* Ruiz et Pav. Bull. Soc. bot. France **97**: 196-197.

— 1952. Embryologie des Scrofulariacées. Développement de l'albumen et de l'embryon chez le *Teedia lucida* (Soland.) Rud. Bull. Soc. bot. France **99**: 266-268.

— 1953. Embryologie des Scrofulariacées. Développement de l'albumen et de l'embryon chez le *Digitalis purpurea* L. Phytomorphology **3**: 168-172.

— 1954. Le développement de l'albumen et de l'embryon chez le *Tetranema mexicanum* Benth. (Scrofulariacées). Bull. Soc. bot. France **101**: 233-235.

Håkansson, A. 1926. Zur Zytologie von *Celsia* und *Verbascum*. Lunds Univ. Årssk. Nr. F. avd. 21 (2,10).

Persidsky, D. 1934. On the development of the endosperm and haustorium in *Linaria genistaefolia* L. Bull. Jard. bot. Kieff **17**: 11-18.

Schmid, E. 1906. Beiträge zur Entwicklungsgeschichte der Scrophulariaceae. Beih. bot. Zbl. **20**A: 175-290.

Souèges, R. 1948. " Embryogénie et classification. 3è fasc. Essai d'un système embryogénique. Partie spéciale: Ire période du système." Hermann et Cie, édit., Paris.

Wettstein, R. von 1891. Scrophulariaceae; dans Engler, A. et Prantl, K. Die natürlichen Pflanzenfamilien, Leipzig, **4**. Teil, 3. Abt. **b**: 39.

Svensson, H. G. 1928. Zur Entwicklungsgeschichte der Blüten und Samen von *Limosella aquatica* L. Svensk bot. Tidskr. **22**: 465-476.

A NEW TERRESTRIAL SPECIES OF *VAUCHERIA* FROM SOUTH INDIA

N. A. ERADY
University College, Trivandrum, India

The alga forming the subject of this communication was found growing extensively on exposed, moist, clayey ground near the Paravoor backwater lake at Mayyanad, Travancore-Cochin State, in May 1953, and has been repeatedly observed throughout the subsequent months of the year. Growth was quite luxuriant during May-July and September-October. In November, when the water in the lake became slightly brackish by the entry of sea-water through a temporary opening in the sand-bar separating the sea from the lake, the alga showed stunted growth and progressive deterioration of the portions above ground.

To a casual observer, the alga recalls a dense growth of tiny young moss plants. The plant body is fixed to the substratum by means of a well-developed system of sparsely branched, colourless rhizoidal filaments (Figs. 1, 2). They are 7-60 μ broad and may penetrate the clayey soil to a depth of nearly 10-50 mm. The green aerial system (Figs. 1-4) is normally erect with a few branches originating from the base and reaching a length of approximately 2-10 mm. But when there is abundance of water in the substratum, or when the alga is completely submerged under water, as it usually happens during the rainy months of the year, there is a marked tendency for rapid growth and elongation of the portions above ground. The branches are 25-97 μ broad, cylindrical and with rounded apex.

The wall of the tubular coenocytic thallus consists of two layers. Of these the outer layer is readily stained by aqueous solutions of both ruthenium red and methylene blue which indicates its pectic nature. The amount of pectic substance is, however, greater in the aerial filaments than in the rhizoidal filaments. Treatment of the alga with several drops of chlor-zinc-iodide for about 15 minutes imparts a bluish-violet colour to the inner wall layer and this reaction shows that this layer is composed of cellulose. The material stains best if left in water for some hours before staining. A large number of discoid chloroplasts without pyrenoids lie imbedded in the thin layer of cytoplasm lining the wall. Under normal conditions plenty of fat globules are seen inside the filaments.

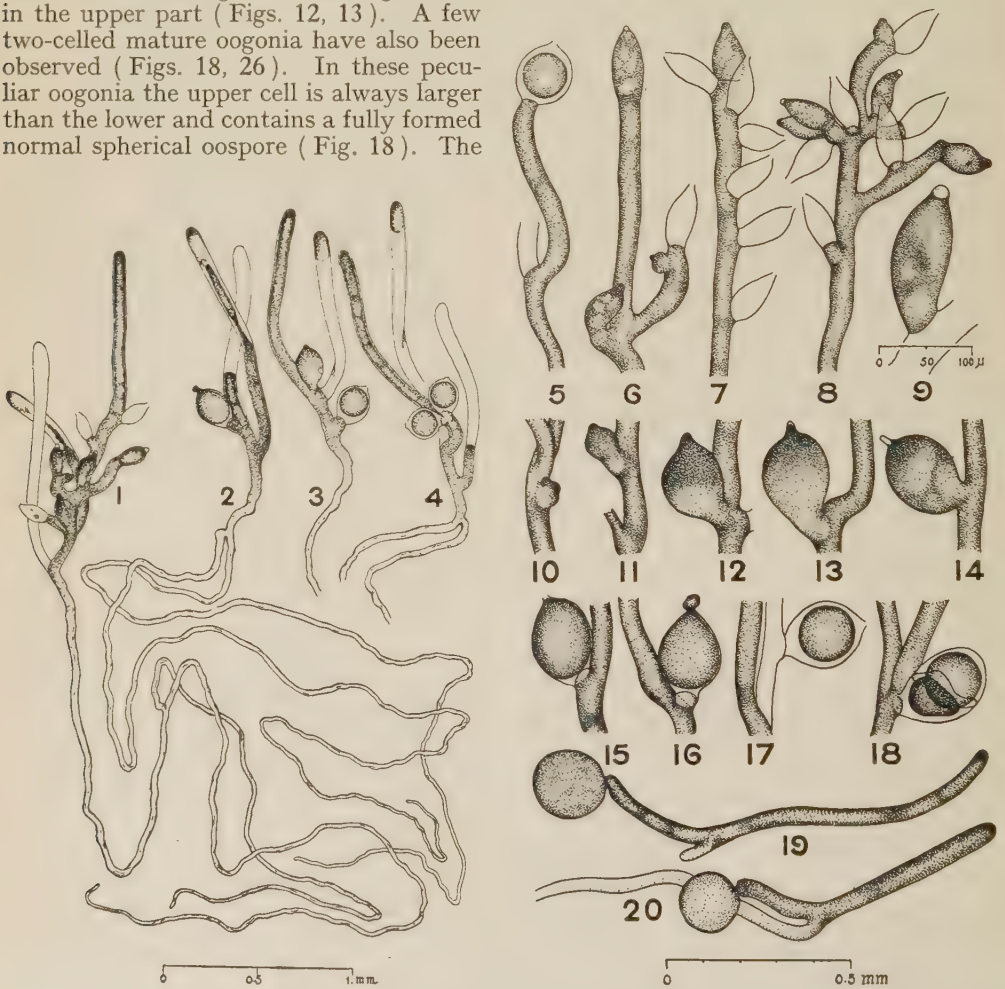
The formation of asexual and sexual reproductive structures is restricted to the aerial portions of the filaments (Figs. 1-4, 21-26). Asexual spores resembling zoospores, aplanospores and akinetes have been repeatedly observed. Details regarding their mode of formation, liberation and development have not yet been followed.

Sexual reproduction is of frequent occurrence. The plants are strictly dioecious. The male plants (Figs. 1, 6-8, 21, 22) are often much more branched than the female (Figs. 2, 3, 23, 25, 26). Usually the sessile, elongate, ovate or broadly lanceolate antheridia are produced in large numbers either laterally (Figs. 7, 8) or at the ends of short or long branches (Figs. 1, 6, 8). They are 103-193 μ long and 44-75 μ broad. When mature, the antheridium opens by an apical pore (Figs. 6-8) which is formed by the gelatinization of the blunt hyaline beak (Fig. 9). In the male plants proliferation is very common and this results in the formation of clusters of antheridia (Fig. 8).

The principal features in the development of the oogonium appear from Figs. 10-17. They are generally sessile, rarely shortly stalked (Fig. 27), 133-223 μ long

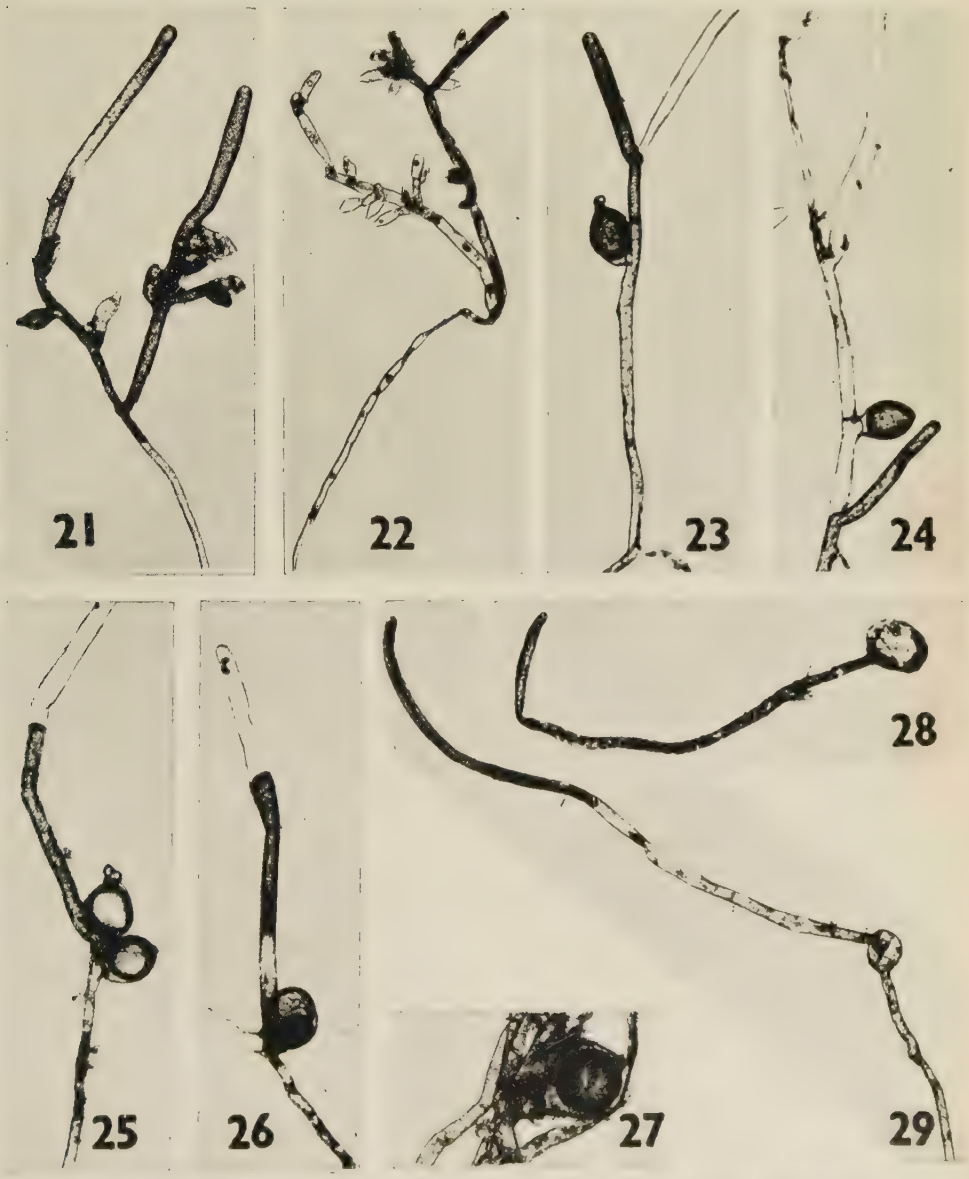
and 103-178 μ broad. The number and position of the oogonia may vary according to individual plants (Figs. 2-5, 23-26). But the type that is represented in Fig. 5 is rather rare. They are either spherical or ovate and with a terminal, hyaline beak (Fig. 14) which gelatinizes at maturity leaving an opening in the oogonial wall (Fig. 15). The maturing oogonium has a peculiar appearance due to the accumulation of the dark green colouring matter in the upper part (Figs. 12, 13). A few two-celled mature oogonia have also been observed (Figs. 18, 26). In these peculiar oogonia the upper cell is always larger than the lower and contains a fully formed normal spherical oospore (Fig. 18). The lower cell also contains an oospore-like body which is completely free from the oogonial wall and deeper in colour (Figs. 18, 26). But it is much smaller than the normal oospore and is either hemispherical or slightly irregular in shape.

The oospore (Figs. 3-5, 17, 27) is perfectly spherical, light brown, 89-149 μ in diameter and with a very slightly thick wall. The oogonial wall is completely



FIGS. 1-20 — *Vaucheria mayyanadensis* sp. nov. Fig. 1. Entire male plant with antheridia. Fig. 2. Entire female plant with mature oogonium. Figs. 3, 4. Aerial portions of female plants showing lateral oogonia. Fig. 5. Aerial portion of female plant with terminal oogonium. Figs. 6-8. Aerial portions of male plants showing antheridia in various stages of development. Fig. 9. External view of mature antheridium showing the blunt hyaline beak. Figs. 10-16. Stages in the development of oogonium. Fig. 17. Oogonium with spherical oospore. Fig. 18. A two-celled mature oogonium. Figs. 19, 20. Early and late stages in germination of the oospore showing origin and development of rhizoidal and aerial systems.

free from the oospore and adheres to it when it breaks away from the pedicel, and later gelatinizes and disappears. Its capacity to withstand extreme dry conditions is remarkable. The oospore invariably germinates only after a period



FIGS. 21-29 — *Vaucheria mayyanadensis* sp. nov. Fig. 21. Mature male plant showing antheridia in various stages of development. × 34. Fig. 22. Old male plant with a large number of empty antheridia in clusters. × 25. Figs. 23-25. Female plants showing mature oogonia. × 34. Fig. 26. Female plant with two-celled oogonium. × 34. Fig. 27. Part of a female plant with a shortly stalked oogonium containing a mature spherical oospore. × 56. Fig. 28. Early stage in the germination of the oospore showing the origin of the primordium of the rhizoidal system as a lateral outgrowth of the aerial system. × 30. Fig. 29. Late stage in germination of oospore showing the origin of rhizoidal system directly from the oospore. × 30.

of rest. During germination (Figs. 19, 28) the germ tube comes out through a small opening produced in the wall of the oospore. At first it is colourless, but very soon it becomes green and ultimately develops into the green aerial system. The primordium of the rhizoidal system makes its appearance very early either as a lateral downwardly growing branch of the germ tube (Figs. 19, 20, 28) or as another colourless germ tube originating directly from the oospore just opposite the place of origin of the aerial filament (Fig. 29). In any case, the two systems can be easily recognized even in the juvenile stages of the alga.

In the taxonomic treatments of the genus *Vaucheria* by Wolle (1887), Collins (1909), Heering (1921), Hoppaugh (1930), Randhawa (1939), Dangeard (1939), and in the monograph by Li (1936), the characteristic structural features of the antheridium, such as its shape, manner of opening and the presence or absence of a supporting cell which separates it from the remainder of the filament are taken into consideration in grouping the species into sections. Thus Heering (1921) lists seven sections of which *Woroninia* forms the first. Brown (1929), on the other hand, ignores the division of the genus into sections.

The nature of the antheridium in the species under investigation clearly indicates that it is a member of the section *Woroninia* (see description of section *Woroninia* included in this paper). Heering (1921) describes three species under this section. They are *Vaucheria dichotoma* Agardh, *V. thuretii* Woronin and *V. schleicheri* de Wild. (Syn. *V. nicholsii* Helen J. Brown). A fourth species now attributed to this section is *V. sescuplicaria* Christensen nom. n. (Syn. probab. *V. dichotoma* β *submarina* Lyngbye *pro parte*). Of these *V. schleicheri* has formed the subject of investigation by many workers including Dangeard (1925), Brown (1937), Prescott (1938), Brunel (1947) and Luther (1953). They report this alga from several fresh and brackish water situations both in Europe and in North America. As far as is known, the other three species are strictly marine or brackish water forms. But the new *Vaucheria*, even though capable of tolerating the occasional presence of brackish water in the substratum, is peculiar in showing a marked preference for terrestrial conditions.

Of the four species mentioned above, *V. dichotoma* is usually said to be dioecious, but according to Christensen (1952), the oogonia and antheridia are borne on the same plant, only most usually on different filaments or different parts of one filament. The species under consideration, therefore, represents the only strictly dioecious type of this section.

The new *Vaucheria* differs from all the four previously recorded species by (i) the terrestrial habit, (ii) thallus with a well-marked subterranean colourless rhizoidal system and an erect green aerial system, and (iii) marked dioecism.

These striking differences seem to warrant the establishment of a new species which may be called *Vaucheria mayyanadensis*.

Description

Vaucheria mayyanadensis spec. nov.

Plantae dioicae; thalli filamenta separata in partes aereas atque rhizoidales; filamenta aerea 25-97 μ crassa, fusce viridia, nitentia, sparse ramosa, ut plurimum erecta; filamenta rhizoidea 7-60 μ crassa, subterranea, incolora, sparse ramosa, atque ter ad decies longiora quam filamenta aerea. Chromatophoris discus, pyrenoidibus carentibus. Antheridia 103-193 μ longa, 44-75 μ lata, singula vel saepius aggregata, sessilia vel terminalia insidentia brevibus longisve ramis lateralibus, ovata vel late lanceolata, apice terminali obtuso, hyalino, ad maturitatem gelatinoso evadente atque producente porum terminalem. Oogonia 133-223 μ longa, 103-178 μ lata, ut plurimum sessilia, raro breviter stipitata, saepissime singula, rarius bina vel plura, axi longo generatim angulum efformante cum erecto filamento, raro eidem parallelo vel cum eodem angulum rectum efformante, sphaerica vel ovata, apice terminali hyalino, ad maturitatem gelatinoso evadente; zygosporae sphaericae, 89-149 μ diam., semitransparentes, penitus liberae persistentes in oogonio, zygosporarum parietibus crassis,

brunnee maculatis, oogonii parietibus persistentibus atque zygosporam includentibus, tandem gelatinosis evadentibus.

Typus lectus in argillosis ad ripas Paravoor in loco Mayyanad, in Statu Travancore-Cochin, mense maio 1953, a N. A. Erady et K. Rajappan, et positus in Laboratorio Botanico, in Collegio Universitatis, Trivandrum, in India meridionali.

Vaucheria mayyanadensis sp. nov.

Plants dioecious; thallus filaments distinguished into aerial and rhizoidal portions; aerial filaments 25-97 μ thick, deep green, shiny, sparsely branched, usually erect; rhizoidal filaments 7-60 μ thick, subterranean, colourless, sparsely branched and three to ten times as long as the aerial filaments. Chromatophore discoid, without pyrenoids. Antheridia 103-193 μ long, 44-75 μ broad, solitary or often in clusters, sessile, lateral or terminal at the end of short or long branches, ovate or broadly lanceolate, terminal beak blunt, hyaline, gelatinizing at maturity and forming a terminal pore. Oogonium 133-223 μ long, 103-178 μ broad, generally sessile, rarely very shortly stalked, frequently one, less often two or rarely more, long axis generally making an angle with or rarely parallel to or at right angles to the erect filament, spherical or ovate, terminal beak hyaline and gelatinizing at maturity; oospore spherical, 89-149 μ in diameter, semi-transparent, completely remaining free inside the oogonium, oospore wall very slightly thick, brown, spotted, oogonial wall persisting around the oospore and getalinizing later.

On moist clayey ground near banks of Paravoor backwater lake at Mayyanad, Travancore-Cochin State, in May 1953. Collected by N. A. Erady and K. Rajappan. The type material of *V. mayyanadensis* which bears No. 1025 in the author's collection is deposited in the Botany Laboratory, University College, Trivandrum, South India.

The new species resembles *V. thuretii* and *V. sescuplicaria* in many respects and is undoubtedly a closely related form. The features of similarity include: (i) the approximate uniformity in the width of the green filaments, (ii) narrowness of the antheridium, (iii) spherical shape of the oospore, and (iv) free nature of the oospore from the oogonial wall.

In describing *V. sescuplicaria*, Christensen (1952) states that it resembles *V. dichotoma* in most respects. But it is clear from the description and diagrams given in the text that they differ in most of the important characters including the width of the thallus, the size, shape and position of the sex organs and nature of the oospore. Moreover, the points of resemblance suggested by him are so meagre that it is difficult to understand their close similarity. Since *V. dichotoma* is closely related with *V. schleicheri* — a point which has been stressed recently by Luther (1953) — and as the latter shows no features of close similarity with *V. sescuplicaria*, it would only be more appropriate to group *V. sescuplicaria* with *V. mayyanadensis* and *V. thuretii* than with *V. dichotoma*.

Consequent upon the inclusion of this new species in the section *Woroninia*, it has seemed desirable to incorporate in this paper a description of this section, which is mainly based upon the work of Heering (1921), and a key to the species of the genus *Vaucheria* included in it.

Section *Woroninia* Solms-Laubach — Plants monoecious or dioecious. Antheridium perpendicular to or making an angle with or parallel to the spreading or erect filament, solitary or in clusters, sessile, lateral or terminal, lanceolate, oblong, pear-shaped, ovoid, always opening by a single papillate terminal round pore. Oogonium often radially symmetrical, sessile or shortly stalked, with or without a prominent beak and opening by an apical pore. Wall of zygote brownish.

KEY TO THE SPECIES OF *Vaucheria* SECT. *Woroninia*

Green filaments usually above 80 μ broad.

1. *V. dichotoma* (L.) Agardh
   Antheridium 75-153 μ broad and more or less perpendicular to the thallus.
2. *V. schleicheri* de Wild.
   Antheridium 39-80 μ broad and making a more or less acute angle with the thallus.

Green filaments usually up to 80 μ broad. Plants monoecious.

3. *V. thuretii* Woronin
   Antheridium and oogonium more or less making an angle with the thallus; oogonium always independent of the antheridium.
4. *V. sescuplicaria* Christensen nom.n.
   Antheridium and oogonium more or less perpendicular to the thallus; oogonium always developing at the base of an antheridium.

Plants dioecious.

5. *V. mayyanadensis* Erady sp. nov.

Summary

A species of *Vaucheria*, found growing on the banks of the backwater canal in Travancore-Cochin State, differs significantly in many characters from other recognized species of the genus and is considered as a new species. Attention is drawn to the terrestrial habit and the dioecious nature of the plant body. The name *Vaucheria mayyanadensis* sp. nov. is proposed for the reception of this alga and the diagnostic features of the species are described. A description of the section *Woroninia* and a key to the species of *Vaucheria* of this section are also given. As here defined, the typical growth habit, dioecism, size, shape and mode of dehiscence of the antheridium and the nature of the oospore are the distinctive features of the species.

In conclusion the writer wishes to acknowledge his indebtedness to the late Professor F. E. Fritsch for helpful suggestions in the identification and study of the new species and to Professors Gilbert M. Smith and M. O. P. Iyengar for kindly going through and criticizing the manuscript. Thanks are also due to Dr. A. Abraham for encouragement and advice and to Rev. Fr. H. Santapau for kindly putting the description of the species into Latin.

Literature Cited

Brown, Helen, J. 1929. The algal family Vaucheriaceae. Trans. American Micr. Soc. **48**: 86-117.

— 1937. A new species of *Vaucheria* from Northern Michigan. Trans. American Micr. Soc. **58**: 283-284.

Brunel, Jules. 1947. *Vaucheria schleicheri* de Wild. in North America. Contr. Gray Herb. **165**: 62-69.

Christensen, T. 1952. Studies on the genus *Vaucheria*. I. Bot. Tidsskr. **49**: 171-188.

Collins, F. S. 1909. "The Green Algae of North America." pp. 79-490.

Dangeard, P. 1925. Note sur le *Vaucheria schleicheri* de Wild. dans le Lac d'Annecy. Le Botaniste **16**: 271-274.

— 1939. Le genre *Vaucheria* specialement dans la region du sud-ouest de la France. Le Botaniste **29**: 183-265.

Heering, W. 1921. "Chlorophyceae. IV. Siphonocladiales, Siphonales (in Pascher's Die Süsswassarflora Deutschlands, Österreichs und der Schweiz)." Heft **7**: 69-99.

Hoppaugh, K. W. 1930. A taxonomic study of the species of the genus *Vaucheria* collected in California. American J. Bot. **17**: 329-347.

Li, L. C. 1936. A monograph of the algal genus *Vaucheria* in China. Bull. Fan Inst. Biol. Peking (Bot.) **7**: (3).

Luther, H. 1953. *Vaucheria schleicheri* de Wild. neu fur Nordeuropa. Memor. Soc. Fauna Flor. fenn. **28**: 32-40.

Prescott, G. W. 1938. A new species and a new variety of the algal genus *Vaucheria* de Candolle with notes on the genus. Trans. American Micr. Soc. **57**: 1-10.

Randhawa, M. S. 1939. Genus *Vaucheria* in Northern India. Arch. Protistenk **92**: (3).

Wolle, F. 1887. "Fresh Water Algae of the United States." pp. 146-154.

STUDIES OF FLORAL MORPHOLOGY IN THE ERICALES — III. ORGANOGRAPHY AND VASCULAR ANATOMY IN SEVERAL SPECIES OF THE ARBUTEAE[1]

BARBARA F. PALSER
University of Chicago, Chicago 37, Ill., U.S.A.

Introduction

The subfamily Arbutoideae of the family Ericaceae offers a fertile field for investigation of the various aspects of floral morphology. Four papers have already appeared: three dealing with members of the tribe Andromedeae (Palser, 1951a, 1951b, 1952), and one with three species of *Gaultheria* (Chou, 1952). The present paper deals with the organography and vascular anatomy of the flower of twenty-eight species and one variety in the tribe Arbuteae.

The tribe Arbuteae has been considered by some systematists to have as few as two genera — *Arbutus* and *Arctostaphylos*. By others, the number has been increased by segregation from *Arctostaphylos* and the following genera used: *Comarostaphylis*, *Xylococcus*, *Xerobotrys*, *Daphnidostaphylis*, *Arctous*, *Ornithostaphylis* and *Schizococcus*. Other names occur in the literature, such as *Uva-ursi* and *Mairania*, but these can be reduced to synonomy with one or another of the above. One of the most recent taxonomic treatments of *Arctostaphylos* is that of Adams (1940). He considers as valid segregates *Comarostaphylis*, *Xylococcus*, *Arctous* and *Ornithostaphylis* and maintains the species of *Xerobotrys* and *Schizococcus* in *Arctostaphylos*. According to Adams *Daphnidostaphylis* was a name used only by Klotzsch for most of the species of *Arctostaphylos* which he segregated from *A. uva-ursi*.

Relatively little has been done on details of the various aspects of floral morphology in the Arbuteae. Artopoeus (1903) and Matthews and Knox (1926) described the stamen structure in certain members of the tribe and suggested that all species are quite similar. Payer (1857) has considered floral organogeny in *Arbutus*. Artopoeus (1903) has briefly discussed the developing seed in *Arbutus* and Peltrisot (1904) has done the same for a few species of both *Arbutus* and *Arctostaphylos* (including *Arctous*). Samuelsson (1913) gave some details of the ovule and seed in the Ericaceae; three of the species in which development was followed were in the Arbuteae. In addition to these few studies of aspects of floral morphology, Cox (1948) has described in detail the various characters of the secondary xylem in a number of species of the Arbuteae, including *Arctous* and several species each of *Arbutus* and *Arctostaphylos*. Hagerup (1928) has given the chromosome numbers of four species of the Arbuteae: n = 13 in *Arctostaphylos* (*Comarostaphylis*) *diversifolia*, *Arbutus andrachne* and *A. canariensis* and n = 26 in *Arctostaphylos uva-ursi*.

A single reference has been found containing a description of the vascular pathways in the flower of one of these species, i.e. Doyel's (1942) description of various aspects of floral morphology of *Arctostaphylos viscida*. Copeland in the Pyroleae (1947), Monotropoideae (1939, 1941), Rhododendroideae (1944) and Epacridaceae (1954) and Palser (1951a) in the Andromedeae have found vascular anatomy to be variable and of considerable interest. Descriptions of vascular anatomy are also available for flowers of *Clethra alnifolia* (Clethraceae, Kavaljian, 1952), three species of *Gaultheria*

1. This investigation was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

(Chou, 1952), *Pholisma* (Lennoaceae, Copeland, 1935), two species of the Cyrillaceae (Copeland, 1953), and a few species of *Vaccinium* and *Gaylussacia* (Hancy, 1916).

Material and Methods

Of the many species of the tribe Arbuteae, *Arctous alpina* and *Arctostaphylos uva-ursi* are circumboreal, while the other species of *Arctostaphylos* and its segregates are found in western United States and Central America; a few species of *Arbutus* show a distribution comparable to that of *Arctostaphylos,* but the genus is primarily Mediterranean. The only species occurring within easy access of Chicago is *Arctostaphylos uva-ursi*; consequently collections of material were made by the author on scattered trips, but primarily by friends and colleagues who were kind enough to help. Particular mention should be made of Dr. Herbert F. Copeland who sent his entire collection of Arbuteae which included some seventeen species. He, in turn, had had assistance from his friends and colleagues in getting this collection together.

Table 1 is a summary of the species studied and the source of the material, both as to area and collector. For the species collected by the author the names follow Kearney and Peebles (1951) or Jepson (1925), and for the others the names are those determined by the collectors except for *Comarostaphylis polifolia* which was sent as *Arctostaphylos polifolia.* It will be noted that the greatest concentration of species is in *Arctostaphylos* and its segregates. As yet no source of the Mediterranean species of *Arbutus,* except for those frequently cultivated in the United States, has been available. There is one representative of each of the following segregates of *Arctostaphylos* — *Ornithostaphylis, Xylococcus* and *Arctous,* and two of *Comarostaphylis.* Also, if *Schizococcus* were to be considered distinct, *A. nissenana* has been placed here, and *A. hookeri, A. glauca* and *A. tomentosa* have been placed in *Xerobotrys.*

The collections from Dr. Copeland were largely killed and fixed in Bouin's fluid, most of the others in Conant's modification of Navashin's solution. All were dehydrated in an ethyl alcohol-tertiary butyl alcohol series and embedded in a paraffin-rubber-beeswax mixture. Cross and longitudinal series were cut at 10 μ. The fewest buds, flowers or fruits cut and studied for any one species was ten, the most twenty-four, with an average of sixteen to seventeen per species. All series were stained with Foster's tannic acid-ferric chloride and safranin which had, in earlier studies, been shown to be most satisfactory for investigation of vascular anatomy. All drawings were made with a camera lucida, but the longitudinal diagrams may in part be composites of several adjacent sections of the series.

Observations

The floral organography of those species of the Arbuteae studied is much less variable within the tribe than was true of the species of Andromedeae considered in an earlier paper (1951a). The flower in the Arbuteae is more like that of *Cassiope* or *Enkianthus* than of any others of the Andromedeae. Many features of the vascular supply to the floral organs are also consistent within the tribe, although variation does occur. Accordingly, a generalized description will be given for both organography and vascular pattern; individual descriptions of organography will be dispensed with and any peculiarities that may occur in this area will be added to descriptions of the vascular anatomy of the individual species.

Organography

General — The pedicels are bracteate in many species of Arbuteae. Flowers are essentially pentamerous, pentacyclic with the outer whorl of stamens opposite the petals, actinomorphic, sympetalous, hypogynous and perfect. Variations from the prevalent pentamery occur in all cycles, but most frequently in the carpels. Of over 480 flowers studied, seven showed four sepals, three six; five showed four petals, four six; six showed eight stamens, eleven nine and four eleven. In all the species of *Arctostaphylos* except *A. tomentosa,* and in *Arctous,* the number of carpels

TABLE 1

Species	General area(s) where collection(s) was (were) made	Collector(s)
Arbutus canariensis Duham.	Strybing Arboretum, Golden Gate Park, Calif.*	Kavaljian[1]
Arbutus menziesii Pursh	Near Sacramento, Calif.; Eatonville, Wash.; Corvallis, Ore.	Copeland[2], Hawkes[3], Kraus[4]
Arbutus unedo L.	Near Sacramento, Calif.; Barstow, Calif.	Copeland, Kraus
Arbutus unedo var. *rubra Ait.*	Near Kew Gardens, England	Camp[5]
Arbutus xalapensis H. B. & K.	Rancho Benito Juarez, near Oaxaca, Mexico	Carlson[6]
Arctostaphylos alganus†	Alcan Highway, Alaska	Juneau[7]
Arctostaphylos andersonii Gray	Contra Costa Co,, Calif.	Sharsmith[8]
Arctostaphylos canescens Eastw.	Sonoma Co., Calif.	Copeland
Arctostaphylos columbiana Piper	Univ. of Calif. campus	Copeland
Arctostaphylos crustacea Eastw.	Contra Costa Co., Calif.	Sharsmith
Arctostaphylos densiflora Baker	Sonoma Co., Calif.	Copeland
Arctostaphylos glandulosa Eastw.	Glendora Mt. Rd., Calif.; Gould Mesa, Calif.; Upper Tujunga TT, Calif.	Ashby[9]
Arctostaphylos glauca Lindl.	Glendora Mt. Rd., Calif.; north of Bouquet Reservoir, Calif.	Ashby
Arctostaphylos hookeri Don	Between Carmel and Pacific Grove, Calif.	Copeland
Arctostaphylos manzanita Parry	Sonoma Co., Calif.; Univ. of Calif. campus	Copeland
Arctostaphylos nevadensis Gray	Echo Summit, Calif.; Yosemite Nat'l. Park*, Calif.	Copeland, Palser
Arctostaphylos nissenana Merriam	Placerville, Calif.	Copeland
Arctostaphylos patula Greene	Wright's Lake, Calif.; Sequoia Nat'l. Park*, Calif.	Copeland, Palser
Arctostaphylos pringlei Parry	Oak Creek Canyon, Ariz.	Palser
Arctostaphylos pungens H.B. & K.	Rancho Benito Juarez, near Oaxaca, Mexico; Manzanar, east of Oaxaca, Mexico; Oak Creek Canyon, Ariz.	Carlson, Palser
Arctostaphylos stanfordiana Parry	Sonoma Co., Calif.	Copeland
Arctostaphylos tomentosa (Pursh) Lindl.	Between Carmel and Pacific Grove, Calif.	Copeland
Arctostaphylos uva-ursi (L.) Spreng.	Indiana dunes; Benzie Co., Mich.; Black Hills, S.D.; near Juneau, Alaska	Gall[10], Juneau, Olmsted[11], Palser
Arctostaphylos viscida Parry	Sacramento Co., Calif.	Copeland
Arctous alpina (L.) Niedenz.	Eagle Summit, Alaska; Yoho Valley, B.C., Canada	Juneau, Palser
Comarostaphylis diversifolia (Parry) Greene	Los Angeles Co., Calif.; Orange Co., Calif.	Copeland
Comarostaphylis polifolia Zucc.	San Felipe, Chiapas, Mexico; road to Chamula, Cindad las Casas, Chiapas, Mexico	Carlson
Ornithostaphylis oppositifolia (Parry) Greene	So. of Ensenada, Baja California, Mexico	Copeland
Xylococcus bicolor Nutt.	Orange Co., Calif.; San Diego Co., Calif.; no. of Santos Bay, Baja California, Mexico	Copeland

*Collections at Golden Gate Park were made with the permission of the Superintendent of the Arboretum, Mr. Eric Walther, and in the National Parks with the permission of the Park Naturalists.

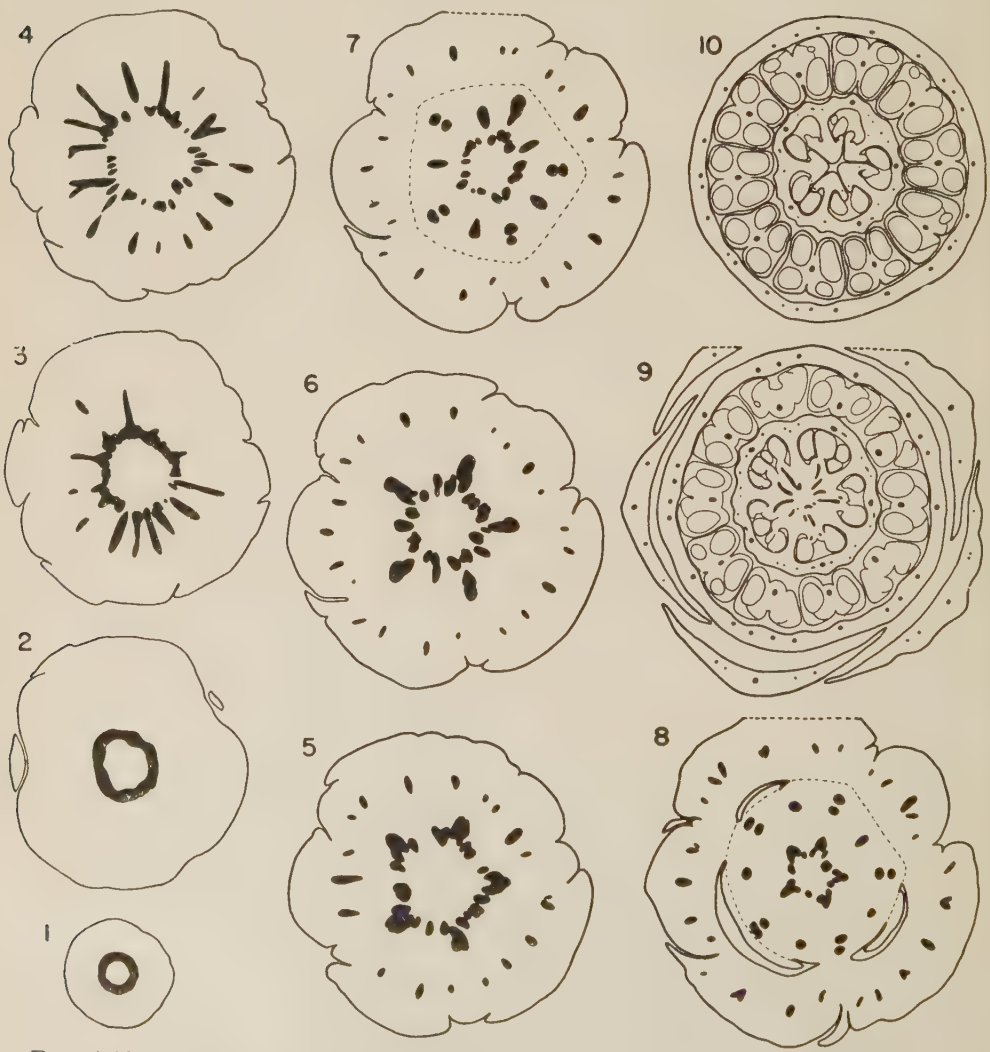
†The material was sent under this name, but it has not been found listed anywhere.

1. Leroy G. Kavaljian, Sacramento State College, Sacramento, Calif.
2. Herbert F. Copeland, Sacramento Junior College, Sacramento, Calif.
3. Margaret J. Hawkes, Hyde Park High School, Chicago, Ill.
4. E. J. Kraus, Oregon State College, Corvallis, Ore.
5. W. H. Camp, Academy of Natural Sciences, Philadelphia, Penna.
6. Margery C. Carlson, Northwestern Univ., Evanston, Ill.
7. Juneau Botanical Club, Juneau, Alaska.
8. Helen K. Sharsmith, Univ. of Calif. Herbarium, Berkeley, Calif.
9. W. Clark Ashby, Calif. Inst. of Technology, Pasadena, Calif.
10. Harold J. F. Gall, Univ. of Chicago, Chicago, Ill.
11. Charles E. Olmsted, Univ. of Chicago, Chicago, Ill.

is more frequently greater than five — ranging upward to nine. In a few instances, the number of carpels was reduced to four. Within a single flower, variations may occur in one cycle only, or in more than one. For example, three flowers were completely tetramerous and in two, all cycles were increased.

The sepals tend to be free from each other although in a number of species they may be undiverged for a short distance at the base (Figs. 49, 50). Their aestivation in the bud is almost always quincuncial (two wholly internal, two wholly external, and one half internal and half external) (Figs. 9, 27, 51). In most species the sepals in the mature flower are turned downwards for a greater or lesser distance from their point of origin before ascending (Figs. 57, 58). If the sepals are small, there may be no ascending portion.

The five petals alternate with the sepals and are undiverged from one another for almost their entire length (Figs. 10, 30, 41). The short free limbs at the tip



FIGS. 1-10 — *Arbutus canariensis.* Diagrams of c.s. series of bud from pedicel to upper half of ovary; sepals omitted in Fig. 10.

most commonly show imbricate aestivation in the bud (one wholly internal, one wholly external, and three half internal and half external), and in the open flower are recurved (Fig. 57). All corollas are essentially urceolate, many strongly so. The base of the corolla in the flower is usually reflexed (Fig. 57) and may show bulges between the sepals at the base.

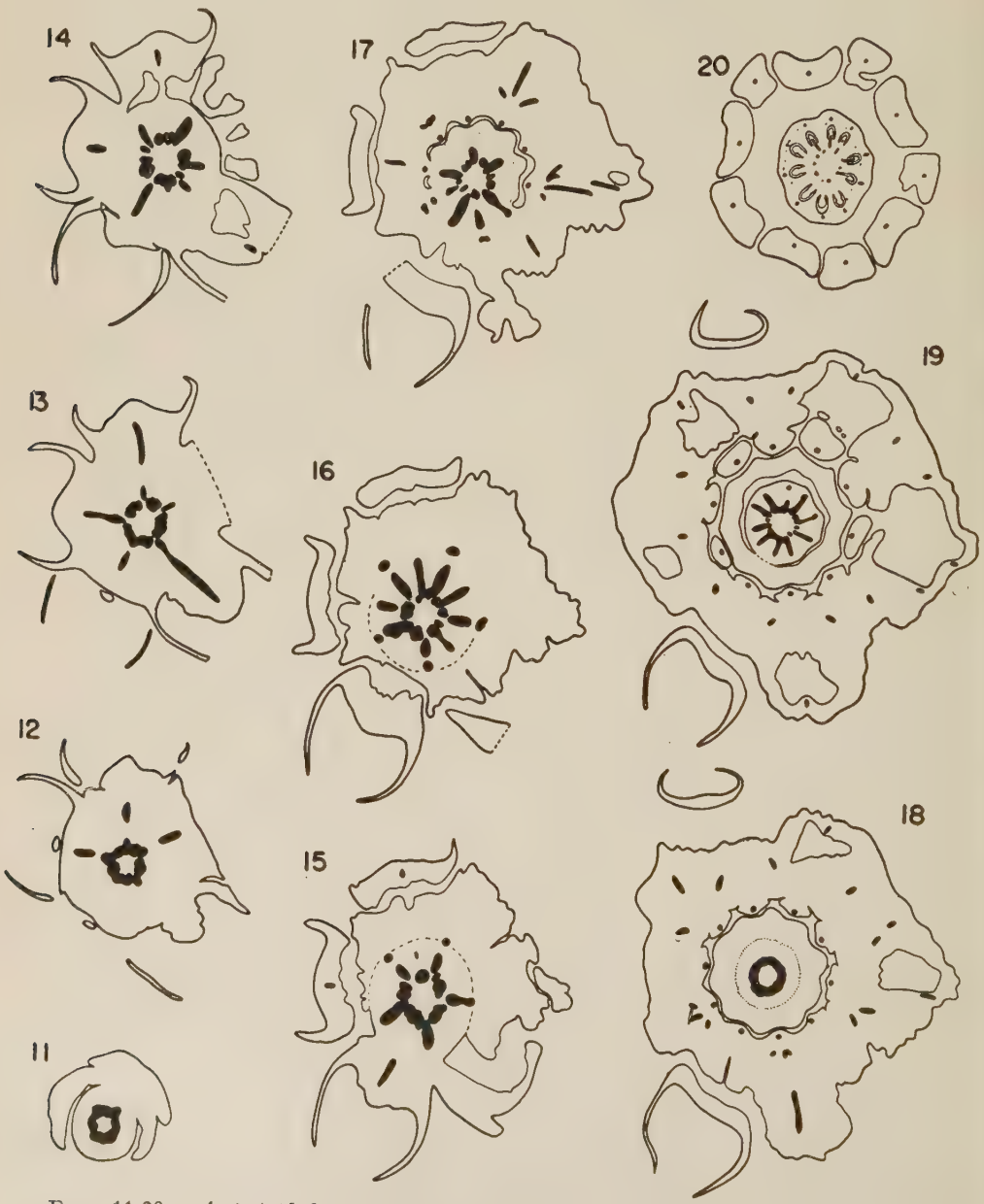
The ten stamens occur in two whorls of five each. The outer whorl, slightly lower in origin, is opposite the petals, while the inner, or upper, is opposite the sepals (Figs. 6-8, 15-17, 26-28, 35-37, 47-49). The filaments are rectangular or oval in cross-section and are considerably expanded for a short distance just above their bases (Figs. 20, 29, 41). The stamens are usually considered not to be epipetalous, but sections show that the tissues of filament and corolla are often undiverged for a short distance, particularly in the sepalad whorl (Figs. 19, 39, 51, 57), and this is reflected in the fact that when the corolla is pulled or falls normally from a flower the stamens go with it. In the bud the anthers are essentially terminal on the filaments and would be considered extrorse (Fig. 56). The two spurs per stamen are attached basally to the inner edge of the anther lobes and extend downwards. The regions of differentiated tissue which will give rise to the pores or slits of the mature anther are at the base and on the outside (Figs. 10, 30). As the flowers approach anthesis, there is a gradual bend at the point of juncture of filament and anther (Fig. 58), with the result that in the open flower the anthers are introrse, the filament attached near the top (Fig. 57), the pores at the apparent apex and the spurs are attached to the upper, outer portion of the anthers and extend downward and outward from this point. In many species the tip ends of the spurs, which may be very long, are turned back upward. In *Arbutus* the connective extends a short way beyond the thecae forming a blunt protuberance (Fig. 56). The anthers contain four thecae, two on each side of the connective (Fig. 10). In older anthers the two thecae on each side become confluent so that each shedding anther has only two pollen sacs. The microspores do not separate following meiosis so that the shedding pollen is in the form of tetrahedral tetrads. In all species studied, the anthers are contained within the urceolate corolla (Figs. 56-58).

The ovary is usually somewhat elongated and does not have the depression at the summit which is characteristic of the Andromedeae (Figs. 56-58). The style is straight and terminates in the slightly lobed stigma just below the level of the corolla opening (Figs. 56-58). In those flowers with five carpels, the locules of the compound pistil occur opposite the petals (Figs. 9, 41); in those with more than five, the locules may be essentially equal in size and evenly distributed, thus showing no direct relation to the members of the other whorls (Fig. 20). In many other species, however, five large locules may occur opposite the petals and the one or more additional carpels are represented by smaller locules which give the appearance of being crowded into the relatively broad septa and are thus opposite one or more of the sepals (Fig. 30). There are broad septa with a central column of tissue, the placentation thus being axile. In *Arbutus* the placenta is two-lobed in the upper portion; the split extends into the central column so that the placentation may thus approach the parietal condition (Fig. 10). The opening continues directly into the stylar canal. In *Arctostaphylos* and its segregates, small openings extend from the summit of each locule into the style where they become confluent, thus forming the stylar canal — the angles of the canal corresponding to the locules, the lobes to the septa. There are approximately ten ovules per carpel in *Arbutus* (Figs. 9, 56), two in *Ornithostaphylis*, and usually only one in the rest of the *Arctostaphylos* complex (Figs. 20, 30, 58). In accordance with the reduced number of ovules the size of the placenta, occurring near the top of the locule, is smaller than that found in most of the Andromedeae. The ovules also are larger than those found in the latter tribe. They are unitegmic, tenuinucellate and essentially anatropous. The ovary surface in *Arbutus*, *Arctostaphylos tomentosa* and *Comarostaphylis* shows multicellular blunt protuberances over the entire surface

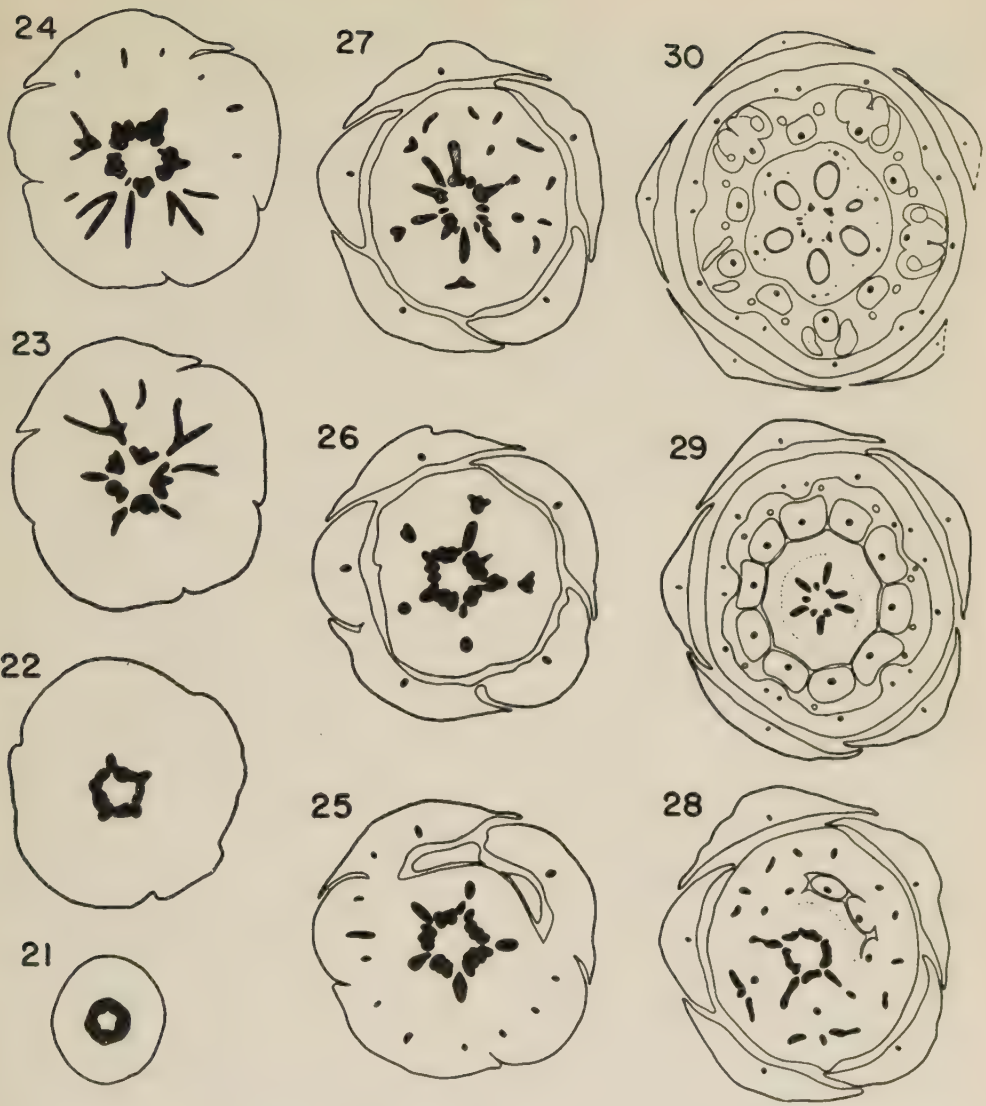
which enlarge greatly in the developing fruit and form the fleshy part of the fruit (Figs. 41, 56, 61).

A well-developed nectary is found in all species. It consists of a mass of glandular tissue at the base of the ovary, that is, between the divergence of the stamens and the opening of the locules, and in most of them extends upward as a free ring of tissue (Figs. 8, 17-19, 28-29, 38-41, 49-51, 56-58).

None of the species is completely glabrous throughout. Hairs of several types are found, either quite thickly or scatter-



FIGS. 11-20 — *Arctostaphylos canescens*. Diagrams of c.s. series of flower from pedicel to upper portion of ovary; corolla and sepals omitted in Fig. 20.



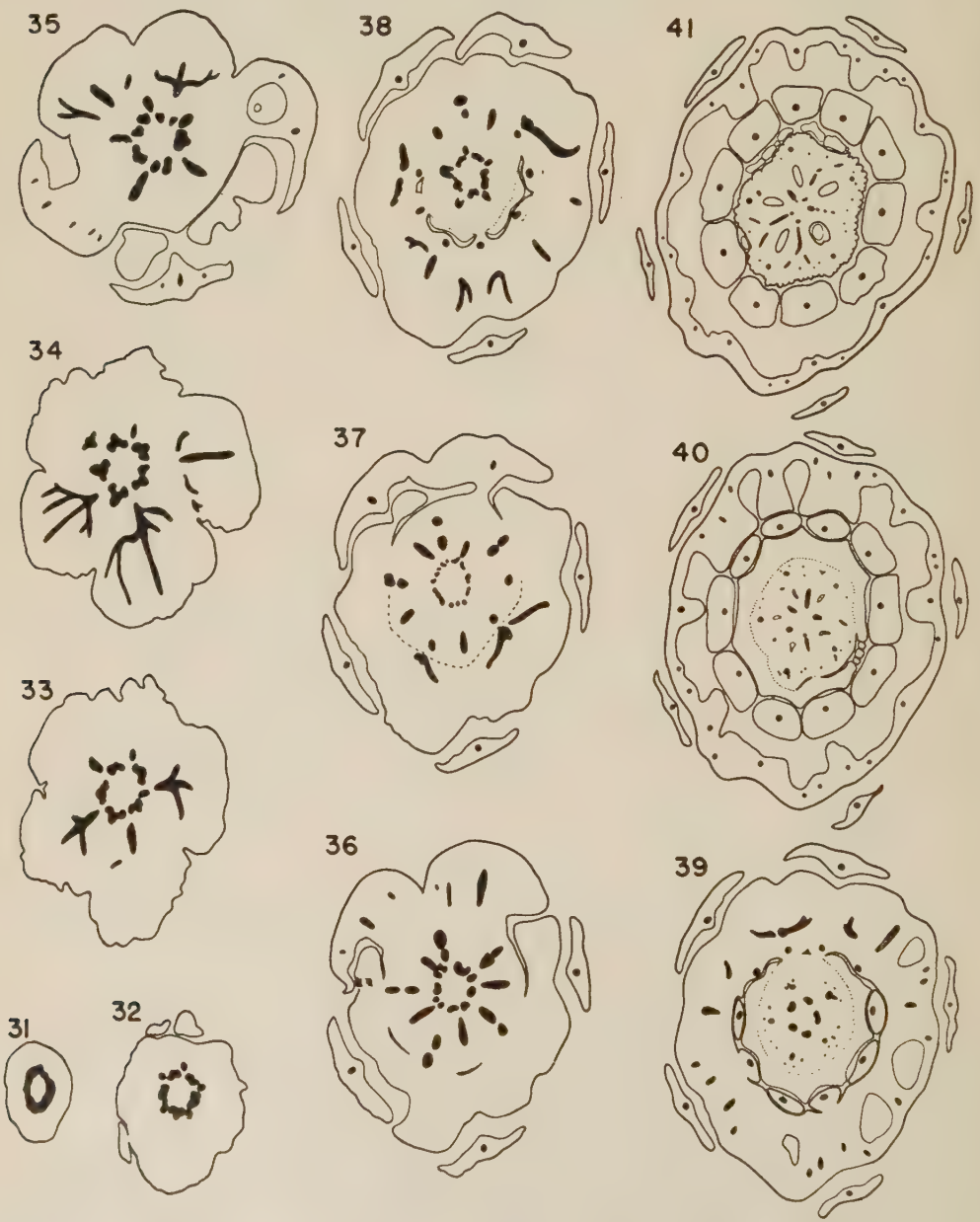
FIGS. 21-30 — *Arctostaphylos nevadensis*. Diagrams of c.s. series of bud from pedicel to middle of ovary.

ingly distributed: unicellular, uniseriate, uniseriate with glandular tip and multicellular with glandular tip. Pedicel (Fig. 60), calyx, petals, filaments (Fig. 64) and/or ovary wall (Fig. 59) may show one or more of these types of hairs. In addition on various organs the epidermal cells, almost all or only scattered ones, may be slightly to highly papillate. This is particularly true of petals near the top of the flower (Fig. 63), anthers, spurs (Fig. 62) and occasionally of filaments and the upper part of the style.

Vascular Anatomy

GENERAL — All species listed have been available for study of the vascular supply to the floral organs, although, in a few for which only fruits were available, only the

traces which occur in the receptacle could be followed for organs other than the carpels. In essentially all species, a complete vascular ring is found in the pedicel (Figs. 1, 11, 21, 31, 42). This is broken by the departure of the trace or traces to the bract or bracts, but the gaps close and the complete ring enters the receptacle where it expands laterally (Figs. 2, 43) and supplies the cycles of floral organs in the following order: sepals, petals, petalad stamens, sepalad stamens, dorsal regions



FIGS. 31-41 — *Arctostaphylos tomentosa*. Diagrams of c.s. series of bud from pedicel to lower part of ovary.

of the carpels, septal regions of the carpels (in the few species which have a septal supply). The vascular tissue remaining in the centre constitutes the ventral supply to the carpels and enters the placentae (Figs. 9, 20). The dorsal carpel bundles supply the style and are located at the angles of the stylar canal.

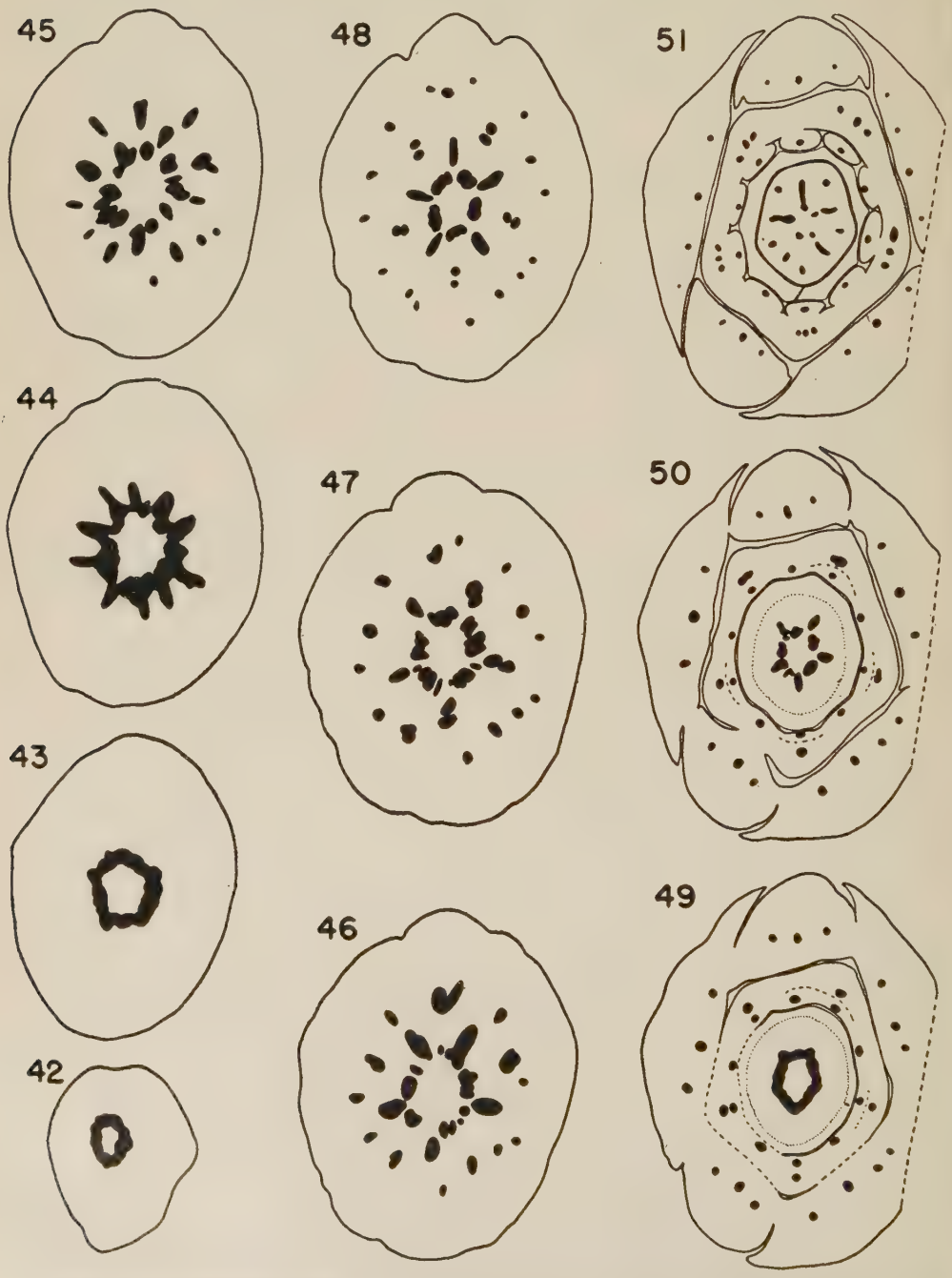
The traces to certain of these organs show some variation among the species studied. Variability occurs in the nature of the sepal supply, the divergence or non-divergence of the petal and petalad stamen traces, the presence or absence of septal carpel traces, and the position of origin of the dorsal carpel traces. The sepal supply varies from three completely independent traces per sepal (Figs. 3-4), each forming its own gap (or in one species, a tendency toward five traces — Figs. 52-55), to a single unbranched trace (Figs. 12-15). Almost a complete range of variation between these two conditions occurs. The most commonly occurring supply is that in which a single trace arises from the cylinder, leaving a single gap, but immediately splits radially into three which enter the base of the sepal (Figs. 23-24). The heaviest supply occurs in *Arbutus*, the least in one species of *Arctostaphylos*. *Xylococcus* (Figs. 44-46), *Ornithostaphylis* and, to a certain extent, *Comarostaphylis* are intermediate between *Arbutus* and most of the species of *Arctostaphylos* in this respect. Where the number of independent traces is less than three per sepal, one lateral may arise independently (Fig. 23), the other either with the median bundle or in common with an adjacent sepal trace — the former in *Arctostaphylos* and its segregates, the latter in *Arbutus*; or both laterals may arise in common with the median bundle as indicated above. In one species a lateral trace may frequently arise as a branch of the adjacent petal trace. Branching of the lateral sepal bundles occurs in many species so that five to nine bundles are found in the base of the sepals (Figs. 34-35). In most species the lateral bundles, and in some even the median bundle, do not extend far. Thus the outer portions of the sepals may contain no vascular tissue.

In both *Arbutus* and in the *Arctostaphylos* complex, there is variability with respect to the matter of divergence or non-divergence of the petal and petalad stamen traces. In no species does the common trace extend far and tangential separation of the two components occurs quite close to the point of origin (Figs. 6-7, 15-16, 35-36, 46-47). In species where the two are independent, the traces to the petalad stamens normally arise before the closure of the petal gaps (Fig. 26). Each trace originates either as a double bundle, half from each side of the gap, which quickly becomes single, or as a single bundle from one side of the gap.

Septal carpel traces are found in those segregates of *Arctostaphylos* listed under separate generic names in this paper (Fig. 51), in *A. tomentosa* (Fig. 41) and *A. alganus*, and in *Arbutus xalapensis* and *A. unedo* var. *rubra*. In the latter the number of such traces is often less than the customary five. These traces arise from the central cylinder in the plane of the median sepal bundles and pass upward in the ovary wall at the outer edge of the septa and fade out at the top of the ovary. In the other species the carpel wall is supplied by branches of the dorsal carpel bundles, although some of them, both in *Arbutus* and *Arctostaphylos*, may occasionally show one or two traces arising from the central cylinder and following a course in the septal region.

The dorsal carpel supply shows essentially two methods of origin. In *Arbutus*, in the segregates, and in many of the species of *Arctostaphylos* five bundles arise in the planes of the petals and supply the dorsal regions of five carpels (Figs. 8, 38-39, 50). If the number of carpels is higher, as it is in most species of *Arctostaphylos*, the extra one or more dorsal traces arise between two or more of these five bundles (Fig. 29), or rarely by a radial splitting of one of them, and pursue a course in the dorsal region of the extra locules which, as indicated earlier, appear to be crowded into one or more of the septa of a normal five-carpellate ovary. In addition to occurring in the same plane, these supernumerary dorsal bundles show a further similarity to septal bundles: they arise normally at a slightly higher level

than do the five main dorsal bundles. In eight of the species of *Arctostaphylos* the vascular tissue remaining above the departure of the stamen traces forms an essentially complete ring (Fig. 18) and from this ring relatively equally spaced



FIGS. 42-51 — *Xylococcus bicolor*. Diagrams of c.s. series of bud from pedicel to base of ovary.

traces arise and supply the dorsal regions of the carpels of that particular flower (Fig. 19). Within each of these species the number of carpels in a flower may be from five to nine, although it is most frequently more than five; the plane of origin of the traces thus usually bears no recognizable relation to the plane of origin of any of the traces to other floral organs.

Small vascular strands extend from the bundles in the carpel wall to the edge of the nectariferous tissue in essentially all species. These normally do not continue into what would be considered the nectary proper.

The vascular supply to the other cycles of floral organs is similar in all species studied. Each petal is supplied with a single trace which leaves a gap in the central cylinder (Figs. 5-7, 14-16, 24-26, 34-36, 45-47). This bundle branches into three in the base of the corolla at about the level it becomes free from the receptacle (Figs. 17-18, 26-28, 37-39, 50-51). In some species the laterals may branch, particularly near the summit of the corolla, one or more times (Fig. 10).

Each stamen, whether petalad or sepalad, receives a single bundle which extends through the filament and the connective between the thecae — upright in non-inverted anthers (Fig. 58) and with a sharp bend in the inverted ones (Fig. 57). In *Arbutus* the strand extends into the projection of the connective beyond the thecae (Fig. 56). As indicated above, the trace to the petalad stamen may be independent or may be undiverged from the petal trace for a short distance. In all species the trace to the sepalad stamen is independent in its origin at a point above the closure of the median sepal gap (Figs. 6-7, 16-17, 27-28, 36-37, 48).

INDIVIDUAL SPECIES — *Arbutus canariensis* Duham. (*A. longifolia* Andr., *A. procera* Soland)[2] (Figs. 1-10, 56, 61-64) — Each sepal receives three traces which are usually independent in origin, although occasionally laterals of adjacent sepals may have a common origin. The laterals usually branch shortly after their origin so that five to nine bundles are found in the base of the sepals. The lateral petal bundles divide shortly after the corolla becomes free from the receptacle. Petal and petalad stamen bundles are undiverged for a short distance.

Arbutus menziesii Pursh (*A. procera* Dougl., *Comarostaphylis glauca* Baker, *Arbutus texana* Buckl.)[2] — Each sepal is supplied by three traces which are usually independent in origin; the laterals branch and rebranch close to their point of origin so that more than five traces are normally found in the base of each sepal. These do not extend far. The lateral petal traces also divide fairly near their point of origin. The petalad stamen trace is independent, arising as a double strand from either side of the petal gap. Septal carpel bundles occasionally occur.

Arbutus unedo L. (*A. crispa* Hoffmgg., *A. integrifolia* Sims, *A. intermedia* Heldr. *ex* Nym., *A. laurifolia* L. f., *A. microphylla* Hort., *A. serratifolia* Salisb., *A. turbinata* Pers. *ex* Reichb., *A. nothocomaros* Heldr. *ex* Nym.)[2] — Each sepal receives basically three bundles which are most frequently independent in origin. Adjacent laterals occasionally arise together. The laterals divide so that five to seven traces reach the base of a sepal, but none of the bundles extends far. The petalad stamen traces are independent in origin, arising sometimes as a double strand from both sides of the petal gap, sometimes as a single strand from one side of the gap.

Arbutus unedo var. *rubra* Ait. (*A. unedo* var. *croomii* Hort.)[3] — This variety corresponds quite closely to *Arbutus unedo*, except that there are usually three or more septal bundles, although there may not be the expected number of five.

Arbutus xalapensis H. B. & K. (?*A. densiflora* H. B. & K., ?*A. petiolaris* H. B. & K., *A. laurifolia* Lindl., *A. rubescens* Bertol., *A. xalapensis pubescens* Benth., *A. varians* Benth., ?*A. floribunda* Mart. & Gal., *A. macrophylla* Mart. & Gal., *Comarostaphylis rubescens* Klotzsch, *A. texana* Buckl., *Arctostaphylos rubescens* Hemsl., *Arbutus xalapensis texana* A. Gray, ?*A. xalapensis petiolaris* Loesener)[4] (Figs. 52-55) — The vascular supply to each sepal is variable,

2. Synonyms taken from "Index Kewensis" (Jackson *et al.*, 1895-1947).

3. Synonyms taken from Rehder (1927).

4. Synonyms taken from North American Flora (Small & Abrams, 1914).

although basically there appear to be five traces. The outer laterals of adjacent sepals frequently arise as a common bundle, often as a double bundle which leaves only a single broad gap, and only occasionally as independent bundles with separate gaps. The median trace and intermediate laterals vary similarly. Occasionally they arise as a single bundle which soon separates to three, but more frequently at least one trace is independent and often all three have a separate origin. The number of gaps left in the central vascular cylinder after the departure of the sepal traces is correspondingly variable. There are never less than ten (five median and five joint outer laterals); most frequently the number is fifteen or more as the result of the independent origin of one or more intermediate laterals per sepal and the occasional independent origin of two adjacent outer laterals. The outer laterals usually branch twice so that at least nine bundles reach the base of a sepal. Any of the inner three bundles may also branch. A peculiar " girdled " region which occurs just above the ovary and below the anthers was observed in the corolla of many of the buds. The petal and petalad stamen traces are undiverged for a short distance. Septal carpel bundles occur.

Arctostaphylos alganus — A single bundle arises from the central cylinder to supply each sepal. This soon divides to three strands which extend into the sepal base. Petal and petalad stamen traces are undiverged for a short distance. Septal carpel traces occur regularly.

Arctostaphylos andersonii Gray (*A. regismontana* Eastw.)[5] (Figs. 57, 59) — The free tips of the petals in this species show a convolute aestivation. The ovary is very hairy. Each sepal is supplied by a single trace from which one or two weak laterals arise. These extend only a short distance. The petalad stamen traces are independent, usually originating as double strands from either side of the petal gap. The dorsal carpel traces arise from a complete ring of vascular tissue, with no reference to other organs unless there are only five carpels.

5. Synonyms taken from Adams (1940).

Arctostaphylos canescens Eastw. (*A. strigosa* Howell, *A. bracteata* Howell)[5] (Figs. 11-20) — Each sepal is supplied by a single unbranched trace. Petal and petalad stamen traces are independent, the latter usually arising from one side of the petal gap. A complete central cylinder of vascular tissue is reformed above the departure of the stamen traces and from this equally spaced dorsal carpel traces arise leaving gaps in the ring.

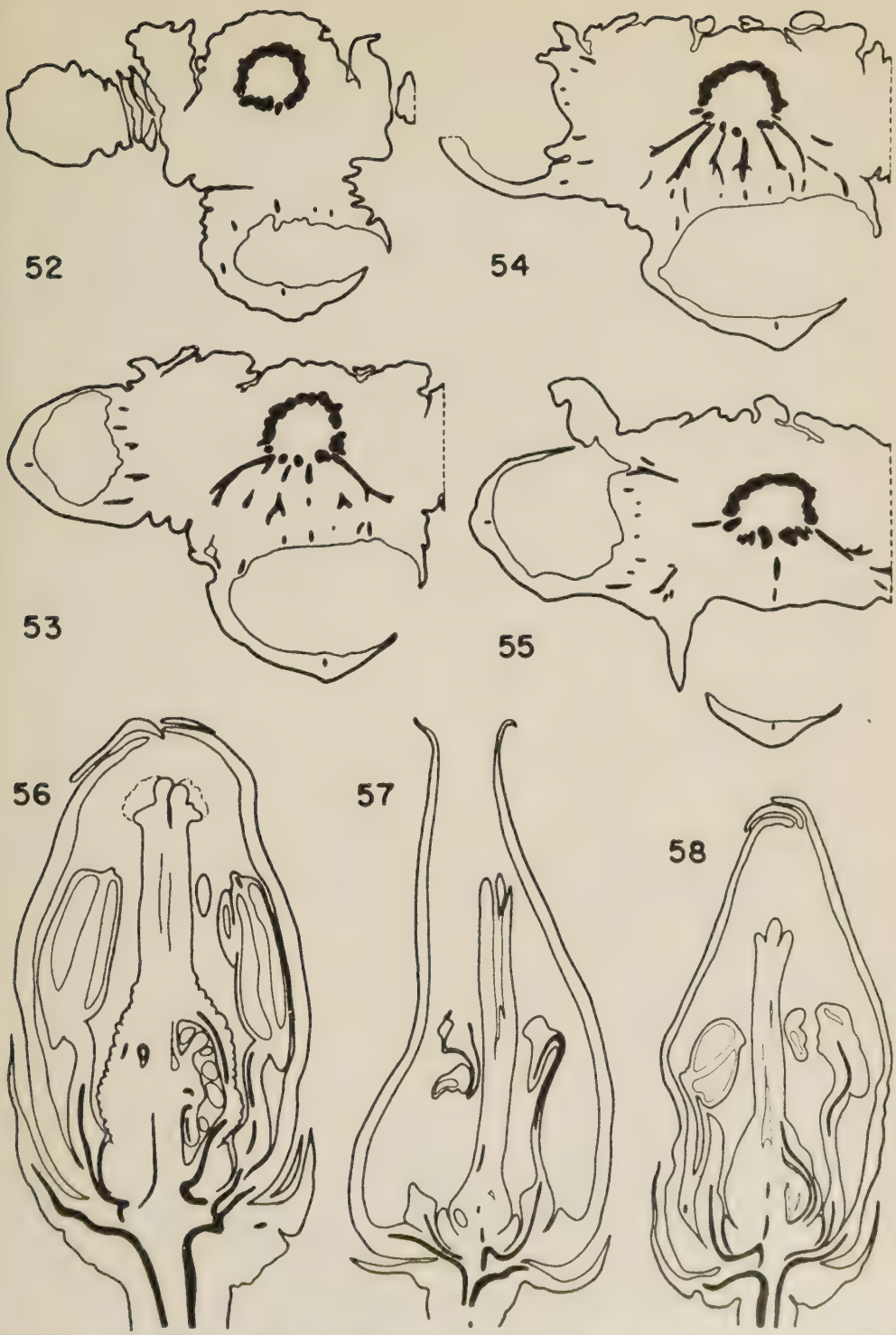
Arctostaphylos columbiana Piper (*Arbutus tomentosa hispida* Hook., *Arctostaphylos setosissima* Eastw.)[5]— The vascular supply to each sepal consists of a single strand which splits to three part way out through the receptacle. Three to five bundles are found in the base of each sepal. The petal and petalad stamen traces are independent, the latter usually arising from one side of the petal gap.

Arctostaphylos crustacea Eastw.[5]— Scattered glandular hairs occur on the pedicel but are almost completely lacking on the sepals. The sepal supply consists of a single trace which splits to three part way out in the receptacle. Only the median bundle extends beyond the portion of the calyx where the sepals are undiverged from each other into the free limb.

Arctostaphylos densiflora Baker[5] — The vascular supply to each sepal arises as a single strand which soon splits into three. The petalad stamen traces arise independently, usually from both sides of the petal gap.

Arctostaphylos glandulosa Eastw. (*A. intricata* Howell)[5] — The ovary is extremely hairy. The sepals in this species have the heaviest vascular supply of any species of *Arctostaphylos* reported in this paper. When the three traces to a single sepal arise as a single bundle, it is as a broad band which immediately separates into its three components. Often at least one of the lateral bundles arises independently. The petal and petalad stamen supply are independent of each other; the latter frequently originates as a double strand. The dorsal carpel bundles arise from an essentially complete vascular ring. Occasional weak septal carpel strands occur.

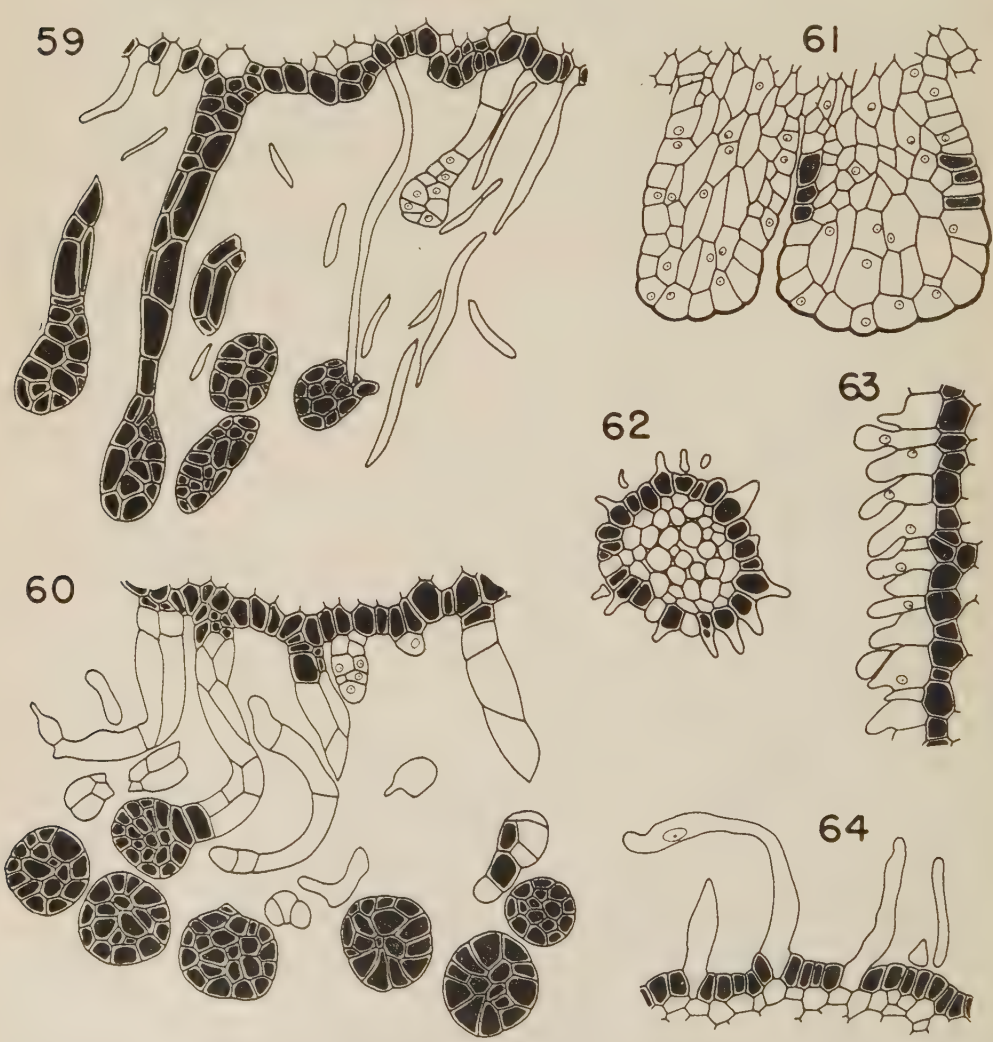
Arctostaphylos glauca Lindl. (*Xerobotrys glaucus* Nutt., *Daphnidostaphylis glauca*



FIGS. 52-58 — Figs. 52-55. *Arbutus xalapensis*, diagrams of slightly oblique c.s. showing vascular supply primarily to one sepal. Fig. 56. *Arbutus canariensis*, diagram of l.s. of bud. Fig. 57. *Arctostaphylos andersonii*, diagram of l.s. of flower. Fig. 58. *Arctostaphylos stanfordiana*, diagram of l.s. of bud.

Klotzsch, *A. glauca* var. *crimicola* Jepson, *A. glauca* var. *puberula* Howell)[5] — The pedicel of this species is heavily covered with glandular hairs. These stop abruptly at the junction of the sepals, which show no hairs. The ovary and style are also covered with glandular hairs. Each sepal receives a single trace which splits to three strands fairly close to its point of origin. The petalad stamen traces arise independently, half of each trace from each side of the petal gap. The origin of the dorsal carpel traces is from a ring of vascular tissue, opposite the petals if there are only five carpels, but with no regular distribution when there are more than five.

Arctostaphylos hookeri Don (*Andromeda venulosa* DC., *Arctostaphylos acuta* Nutt., *Xerobotrys venulosa* Nutt., *Daphnidostaphylis hookeri* Klotzsch, *Arctostaphylos franciscana* Eastw.)[5] — The sepal supply



FIGS. 59-64 — Dermal appendages. Fig. 59. *Arctostaphylos andersonii*, portion of c.s. of ovary wall showing multicellular glandular and unicellular hairs. Fig. 60. *Arctostaphylos viscida*, portion of c.s. of pedicel showing multicellular glandular hairs. Figs. 61-64. *Arbutus canariensis*. Fig. 61. Portion of c.s. of ovary showing blunt protuberances. Fig. 62. C.s. of spur showing papillate epidermal cells. Fig. 63. Portion of c.s. of petal tip showing papillate epidermal cells. Fig. 64. Portion of c.s. of filament showing unicellular hairs.

consists of a single bundle which very soon gives rise to two laterals. The laterals are short and even the median bundle does not extend far. The petal and petalad stamen supply are common for a short distance.

Arctostaphylos manzanita Parry (*A. manzanita* var. *apiculata* Jepson)[5] — The pedicel is very hairy. Each median sepal bundle gives rise to two small laterals well out in the receptacle.

Arctostaphylos nevadensis Gray (*A. pungens* var. Gray)[5] (Figs. 21-30) — The usual vascular supply to a sepal consists of a large bundle which separates into three strands close to the point of origin. For one or two sepals per flower, one lateral may have an independent origin leaving its own gap. The petalad stamen trace is double in its origin, half arising from either side of the petal gap. Many of the lateral petal strands divide near the base of the corolla.

Arctostaphylos nissenana Merriam (*Schizococcus nissenanus* Eastw.)[5] — Each sepal receives three bundles which have been derived from a single trace fairly close to its point of divergence. The petal and petalad stamen traces are undiverged for a very short distance.

Arctostaphylos patula Greene (*A. glauca* Wats. not Lindl., *A. pungens* var. *platyphylla* Gray, *A. platyphylla* Kuntze, *A. obtusifolia* Piper, *A. pinetorum* Rollins)[5] — The vascular supply to the sepals is as in many other species of *Arctostaphylos* — single traces splitting to three a short way from the point of origin with the laterals not extending far. The petal and petalad stamen traces are undiverged for a very short distance.

Arctostaphylos pringlei Parry[5] — The vascular supply to the calyx is quite heavy. Usually a single large bundle, which splits into three almost at the point of origin, supplies each sepal. An occasional lateral bundle, however, may arise independently. The laterals often divide one or more times in the outward course through the receptacle so that five or more bundles may be found in the base of a sepal. The petalad stamen has an independent vascular supply either from both sides or from only one side of the petal gap. The dorsal carpel bundles arise from a complete vascular ring and show no relation to the previous cycles of strands.

Arctostaphylos pungens H. B. & K. (*Daphnidostaphylis pungens* Klotzsch, *A. montana* Eastw.)[5] — The single trace to each sepal gives rise to two short laterals in its outward course through the receptacle. The petalad stamen traces arise independently, usually from one side of the petal gap. The dorsal carpel traces arise from an essentially complete vascular ring and show no regular arrangement with respect to the other whorls.

Arctostaphylos stanfordiana Parry[5] (Fig. 58) — The sepals are each supplied by a single trace which separates into three parts fairly close to its point of origin. The laterals are short. The petal and petalad stamen traces arise very close together, but appear to be actually independent in their origin.

Arctostaphylos tomentosa (Pursh) Lindl. (*Arbutus tomentosa* Pursh, *A. tomentosa nuda* Hook., *Arctostaphylos tomentosa nuda* Lindl., *A. cordifolia* Lindl., *Xerobotrys cordifolium* Nutt., *Xerobotrys tomentosus* Nutt., *Daphnidostaphylis tomentosa* Klotzsch, *D. cordifolia* Klotzsch, *Arctostaphylos vestita* Eastw., *A. glandulosa* var. *vestita* Jepson)[5] (Figs. 31-41) — The main trace to each sepal early divides into three, the laterals branching and sometimes rebranching almost immediately. Occasionally a lateral does not divide but the median strand gives rise to another branch so that at least five bundles reach the base of each sepal. The lateral bundles are short, but the median one extends to the tip of the sepal. Petal and petalad stamen traces arise as a single bundle which separates tangentially into its component parts close to its point of origin. Septal carpel bundles occur regularly.

Arctostaphylos uva-ursi (L.) Spreng. (*Arbutus uva-ursi* L., *Uva-ursi procumbens* Moench., *Mairania uva-ursi* Desv., *Uva-ursi buxifolia* S. F. Gray, *Arctostaphylos officinalis* Wenn. & Grabb., *A. procumbens* Patze, Meyer & Elkan, *Daphnidostaphylis fendleriana* Klotzsch, *A. uva-ursi alba* Cockerell, *Uva-ursi uva-ursi Britton*)[5] — Each sepal trace gives rise to two weak laterals close to the base of the sepal. No trace extends very far.

The petal and petalad stamen traces are undiverged for a short distance. The dorsal carpel traces arise from a complete ring of vascular tissue and show no regular arrangement with respect to the lower whorls of floral organs. Each trace splits into two or three close to its point of departure from the central ring, the laterals travelling in the carpel wall at the edges of the septa.

Arctostaphylos viscida Parry (*A. pulchella* Howell)[5] (Fig. 60) — The upper portion of the pedicel is very densely covered with glandular hairs, but these cease immediately with the expansion of the receptacle and none are found on the calyx. Each sepal receives a single strand. Two or three of the sepal bundles in a flower usually give rise to one, rarely to a second, small lateral trace which does not extend far. The petalad stamen traces originate as double strands, one part from each side of the petal gap. The dorsal carpel bundles arise from an essentially complete vascular ring. This description is in essential agreement with that of Doyel (1942).

Arctous alpina (L.) Niedenz. (*Arbutus alpina* L., *Mairania alpina* Desv., *Arctostaphylos alpina* Spreng.)[6] — The nectary is only an enlargement on the base of the ovary; there is no free ring of tissue. Essentially three traces reach the base of a sepal in this species, but the pattern of their origin is variable. All three may arise by the splitting of a single median trace, or the laterals may arise as branches of the adjacent petal traces, or one lateral may be derived from the median sepal bundle, the other from the adjacent petal strand. Rarely a lateral trace may arise independently from the central vascular ring. The petalad stamen traces have an independent origin, usually from one side of the petal gap. Septal carpel traces occur regularly.

Comarostaphylis diversifolia (Parry) Greene (*Arctostaphylos arguta diversifolia* Parry, *A. diversifolia* Parry)[4] — Usually a single trace departs from the central vascular cylinder to each sepal. An occasional lateral trace is independent. The single trace separates to three close to the central ring and the laterals rebranch so that five to seven bundles reach the base of the sepal although only one, the median, extends very far into the sepal. The petal and petalad stamen traces have a common origin, but separate quite soon. The lateral petal bundles divide in the base of the corolla. Septal carpel bundles occur regularly.

Comarostaphylis polifolia Zucc. (*Arctostaphylos polifolia* H. B. & K.)[4] — Each sepal receives one main trace which branches to three. One of the laterals diverges very close to the point of origin of the common bundle or occasionally arises independently. The latter was true for one sepal in a number of flowers and for three sepals in one of the flowers studied. The sepal laterals often branch so that more than three traces are found in the base of each sepal. Petal and petalad stamen traces are undiverged for a short distance. The petal laterals branch near the base of the corolla. Septal carpel traces arise above the opening of the locules and pursue an upward course in the septa themselves for a considerable distance, giving off branches outward toward the adjacent dorsals. Each placenta becomes double above the attachment of the ovule, the split continuous with the stylar canal.

Ornithostaphylis oppositifolia (Parry) Small (*Arctostaphylos oppositifolia* Parry, *A. salicifolia* Parry, *A. polifolia* Torr.)[2] — Each sepal in this species is supplied by one to three traces which arise independently leaving gaps in the vascular ring. One, or rarely both, lateral traces may arise as branches of the median bundle. The laterals divide during their course through the receptacle once or twice so that seven bundles frequently reach the base of the sepal. Only the median strand extends for any distance. The petalad stamen traces arise essentially independently although very close to the petal traces, usually from one side of the petal gap. Septal carpel traces occur regularly in this species.

Xylococcus bicolor Nutt. (*Comarostaphylis bicolor* Klotzsch, *Arctostaphylos veatchii* Kellogg, *A. bicolor* A. Gray, *A. clevelandi* A. Gray)[4] (Figs. 42-51) —

6. Synonyms taken from Britton and Brown (1913).

The supply to each sepal varies from one to three independent traces, the number per flower averaging around ten. The basic number of traces per sepal is three; if they are not all independent, one or both laterals arise from the median trace close to its point of departure from the vascular ring. Only an occasional lateral trace divides, so three is the most common number of veins in a sepal. Septal carpel traces are regular in their occurrence.

Discussion

A rather lengthy discussion of organography and vascular anatomy in the Ericales was included in the paper dealing with those characters of the Andromedeae (Palser, 1951a) and need not be repeated here except as it relates to the Arbuteae described in this study.

As indicated in the introduction, the Arbuteae show considerably more consistency in organographical features than do the Andromedeae and most of the other tribes of the Ericaceae. They differ from most of the family in the inversion of the anther late, rather than early, during development, in the reduced number of ovules per carpel and in the drupaceous nature of the fruit. The staminal appendage in all species of the Arbuteae studied is a spur arising from the wall of each anther half (only rarely from the connective) at the morphological base of the anther (the apparent apex in the open flower). The Andromedeae, as was reported earlier (1951a), show considerable variation — no appendages, spurs of variable character and awns, also of variable character. The Ericoideae are reported to show no appendages or either spurs and/or awns, and similarly the Vaccinioideae in which an interesting type is a long awn through which the pollen is shed. The only other group of the Ericaceae which has so far been shown to be consistent with respect to staminal appendage is the subfamily Rhododendroideae. Here *no* appendages are found (Copeland, 1944).

Arctostaphylos and all segregates, except *Ornithostaphylis*, are the only genera of the Arbutoideae in which the ovule number is reduced to one per carpel. One is also found in some Vaccinioideae and Epacridaceae. The number ranges upward from this through relatively low numbers such as two in *Ornithostaphylis*, ten in *Arbutus* and a few of the Andromedeae, to as many as two hundred in *Lyonia mariana*. The mature fruit, drupaceous in the Arbuteae, is a capsule in most groups, with the Vaccinioideae, which are epigynous, forming a berry.

While hairs are found on the floral parts of many of the Arbuteae, they are not of the kinds described by Copeland (1944) for various of the Rhododendroideae. Within the Arbuteae there are essentially three types of dermal appendage: papillate epidermal cells; long uniseriate, usually unicellular, hairs; and long multiseriate hairs with ovoid, multicellular, glandular tips. The papillate epidermal cells and unicellular hairs are found in varying density on all species studied. The glandular hairs occur on only seven of the species of *Arctostaphylos* studied. The limited number of species of *Arbutus* available for study here, however, does not allow the generalization that glandular hairs do not occur in this genus. This is not true of the segregates *Ornithostaphylis*, *Xylococcus* and *Arctous* however, as these are monotypic or essentially so. *Comarostaphylis* is more extensive and no generalization can be made from two species. *Comarostaphylis* and *A. tomentosa*, which share the peculiar " granular " ovary wall character with *Arbutus*, also show the same type and similar distribution of hairs.

With respect to the vascular anatomy of the flower, the most variable feature is the supply to the sepal. *Arbutus*, with frequently three independent traces to each sepal, approaches most closely the condition considered to be most primitive and in this respect corresponds to *Leucothoe racemosa* and *L. recurva* in the Andromedeae (Palser, 1951a), *Bejaria* in the Rhododendroideae (Copeland, 1944), *Chimaphila* in the Pyroleae (Copeland, 1947) and *Sprengelia* in the Epacridaceae (Copeland, 1954). The condition found in *Arbutus xalapensis*, which has essentially five traces per sepal rather than three, is probably derived. It could have arisen during evolution by a gradual

branching of the median sepal bundle closer and closer to the point of its departure from the central vascular cylinder until one or both branches may now arise independently. No other species of the Ericales so far reported in the literature shows an increase in number of traces from the primitive three-trace condition; the general tendency is toward reduction. When the number of independently arising traces is reduced, the tendency in *Arbutus* is for the laterals of adjacent sepals to arise together, a condition which has been suggested earlier (1951a) as a possible forerunner of the apparent origin of the sepal laterals from the petal traces. The same type of reduction was seen in several of the Andromedeae such as *Pieris*, *Ampelothamnus* and *Chamaedaphne* (Palser, 1951a). The origin of the sepal laterals from the petal trace, seen in several members of the Rhododendroideae (Copeland, 1944), is found in only one of the species of Arbuteae studied: *Arctous alpina*, and then not regularly. In *Arctostaphylos*, and its segregates other than *Arctous*, the reduction in the number of independent traces to one sepal has been in the direction of a common origin of the laterals with the median bundle. *Ornithostaphylis* and *Xylococcus* normally show about ten gaps in the central cylinder following the departure of the sepal traces, the gaps varying from one to three per sepal. *Comarostaphylis* has a relatively heavy supply but only occasional lateral traces arise independently, more frequently in *C. polifolia* than *C. diversifolia*. In *Arctostaphylos* proper, *A. glandulosa* has the most primitive supply with one lateral trace in one, two, or three sepals showing an independent origin, i.e. it is similar to the condition found in *Comarostaphylis*. The most reduced condition is that exhibited by *A. canescens* in which a single unbranched bundle constitutes the vascular supply to each sepal.

The degree of non-divergence of traces to different whorls of floral organs was not as great for any of the Arbuteae studied as for certain species of the Andromedeae (Palser, 1951a) or the extreme condition in some species of *Vaccinium* (Hancy, 1916). On the other hand, no species showed the extreme of independence that characterizes *Oxydendrum arboreum* (Palser, 1951a). Non-divergence occurred only between petal and petalad stamen bundles in several species or occasionally between lateral sepal and petal bundles in *Arctous*. The outward extent of the common bundle is in no case very great. In no species, however, was there a closure of the petal gap before the departure of the trace to the petalad stamen such as is found in *Oxydendrum*.

In none of the Andromedeae studied (Palser, 1951a) did the vascular supply to the dorsal region of the carpel arise in any plane but that of the median petal bundle. In eight species of *Arctostaphylos*, as here reported, the plane of origin usually had no direct relation to the plane of other floral whorls. In no species of the Andromedeae, however, was the number of carpels characteristically more than five — a condition found in all species of *Arctostaphylos* except *A. tomentosa*.

Within the Arbuteae, *Arbutus* appears to be the more primitive genus on two accounts: the nature of the vascular supply to the sepal and the larger number of ovules. The carpel number is consistently five, however. *Ornithostaphylis* and *Xylococcus* are intermediate in the vascular supply to the sepals, with *Ornithostaphylis* also intermediate with respect to ovule number. Both of these segregates also have five carpels. *Comarostaphylis* and *Arctostaphylos tomentosa* share the peculiar ovary wall character and consistent carpel number with *Arbutus*, and while they have a relatively heavy vascular supply to the sepals, it is not particularly different from that of *A. glandulosa*. *Arctous* is the only genus which shows lateral sepal traces arising from the adjacent petal traces. The carpel number in this species is variable as in *Arctostaphylos*. At the present state of our knowledge of internal morphology there seems to be some slight support for the maintenance of *Ornithostaphylis*, *Xylococcus*, *Comarostaphylis* and *Arctous*. No characters occur, however, which would tend to support the segregation of *Schizococcus* or of *Xerobotrys* unless, of the species in this study which have been placed in the latter genus, *A. tomentosa*

were the only one left in it. Several features of *A. tomentosa* suggest affinities with *Comarostaphylis*. Considerably more work needs to be done on other aspects before any satisfactory conclusions can be drawn, and particular attention needs to be paid to *A. tomentosa*, and probably also to *A. glandulosa*, which appear slightly anomalous in their characters with respect to other members of the genus.

It is difficult to say whether the Arbuteae or Andromedeae should be considered the more primitive tribe in the Arbutoideae. The amount of work on the Gaultheriae is at this time too limited to try to place it with respect to the other two. Both the former tribes show some species with three independent traces to each sepal. The number of ovules per carpel in the Arbuteae is lower than in the Andromedeae, but the degree of non-divergence between whorls is relatively slight in the former tribe when compared to some members of the latter. This suggests, of course, that both tribes have been derived from some unknown common ancestor.

Summary

Twenty-eight species and one variety of the Arbuteae are described with respect to the organography and vascular anatomy of the flower: *Arbutus canariensis, A. menziesii, A. unedo, A. unedo* var. *rubra, A. xalapensis, Arctostaphylos alganus, A. andersonii, A. canescens, A. columbiana, A. crustacea, A. densiflora, A. glandulosa, A. glauca, A. hookeri, A. manzanita, A. nevadensis, A. nissenana, A. patula, A. pringlei, A. pungens, A. stanfordiana, A. tomentosa, A. uva-ursi, A. viscida, Arctous alpina, Comarostaphylis diversifolia, C. polifolia, Ornithostaphylis oppositifolia* and *Xylococcus bicolor*.

The Arbuteae are normally pentamerous, pentacyclic with the outer whorl of stamens opposite the petals, actinomorphic, sympetalous, hypogynous and perfect. Variations occur from the prevalent pentamery in all cycles. The carpels, in *Arctostaphylos* (except A. *tomentosa*) and in *Arctous*, normally number more than five, ranging upward to nine even within a single species. In the open flower the spurred anthers are introrse; the inversion to this position occurs just prior to anthesis. The corolla is urceolate, the number of petals constituting it indicated externally only by the short lobes at the tip. Placentation is essentially axile, with approximately ten ovules per carpel in *Arbutus*, two in *Ornithostaphylis* and one in all other species studied. Each locule has a narrow connection with the stylar canal.

The vascular system in the pedicel forms a complete vascular ring. This broadens in the receptacle and is broken by gaps above the traces to the various organs. The following whorls of traces arise in acropetal order: sepals, petals, petalad stamens, sepalad stamens, dorsal carpel regions — usually in the plane of the petals but in eight species of *Arctostaphylos* with no regular arrangement, septal carpel regions in the few species in which these bundles occur, and the ventral carpel regions or placentae. In several species there is a short region of non-divergence between the petal and petalad stamen traces.

The sepal supply varies from three completely independent traces per sepal, each forming its own gap, as in *Arbutus*, to a single unbranched trace as in *Arctostaphylos canescens*. (*Arbutus xalapensis* shows an increase to five traces though all are not usually completely independent.) Almost a complete range of variation between these two conditions occurs, with the most common being a single trace dividing to three for each sepal. Any reduction in number of traces that may occur in *Arbutus* is accomplished by the common origin of laterals of adjacent sepals. In *Arctostaphylos* and its segregates, the laterals have a common origin with the median bundle.

This study gives some slight support to the segregation of *Arctous, Comarostaphylis, Ornithostaphylis* and *Xylococcus* from *Arctostaphylos*, but supplies no substantiation for the maintenance of *Schizococcus* or *Xerobotrys* as separate genera, unless *A. tomentosa* only, of the species reported on here, were left in *Xerobotrys*.

Arbutus would appear to be the more primitive genus in the tribe, *Arctostaphylos* and *Arctous* more advanced, with *Ornithostaphylis, Xylococcus* and *Comarostaphylis* in an intermediate position.

There is no clear-cut balance of evidence for considering either the Arbuteae or the Andromedeae to be the more primitive tribe in the subfamily Arbutoideae.

The author wishes to express her gratitude to all who gave of their time and energy in collecting the materials which helped to make this publication possible.

Literature Cited

ADAMS, J. E. 1940. A systematic study of the genus *Arctostaphylos* Adans. J. Elisha Mitchell Sci. Soc. **56**: 1-62.

ARTOPOEUS, A. 1903. Über den Bau und die Öffnungsweise der Antheren und die Entwicklung der Samen der Erikaceen. Flora **92**: 309-345.

BRITTON, N. L. & BROWN, A. 1913. "An illustrated flora of the Northern United States, Canada and the British Possessions." Vol. 2. Charles Scribner's Sons, New York.

CHOU, Y. L. 1952. Floral morphology of three species of *Gaultheria*. Bot. Gaz. **114**: 198-221.

COPELAND, H. F. 1935. The structure of the flower of *Pholisma arenarium*. American J. Bot. **22**: 366-383.

— 1939. The structure of *Monotropsis* and the classification of the Monotropoideae. Madroño **5**: 105-119.

— 1941. Further studies on Monotropoideae. Madroño **6**: 97-119.

— 1944. A study, anatomical and taxonomic, of the genera of Rhododendroideae. American Midl. Nat. **30**: 533-625.

— 1947. Observations on the structure and classification of the Pyroleae. Madroño **9**: 65-102.

— 1953. Observations on the Cyrillaceae particularly on the reproductive structures of the North American species. Phytomorphology **3**: 405-411.

— 1954. Observations on certain Epacridaceae. American J. Bot. **41**: 215-222.

COX, H. T. 1948. Studies in the comparative anatomy of the Ericales. II. Ericaceae — subfamily Arbutoideae. American Midl. Nat. **40**: 493-516.

DOYEL, B. E. 1942. Some features of the structure of *Arctostaphylos viscida*. American J. Bot. **29**: 254-259.

HAGERUP, O. 1928. Morphological and cytological studies of Bicornes. Dansk Bot. Ark. **6**: 1-26.

HANCY, A. J. 1916. Vascular anatomy of certain ericaceous flowers. Thesis, Cornell University.

JACKSON, B. D. *et al.* 1895-1947. "Index Kewensis Plantarum Phanerogamarum." 2 vols. and 10 supplements. Clarendon Press, Oxford.

JEPSON, W. L. 1925. "A Manual of the Flowering Plants of California." Berkeley.

KAVALJIAN, L. G. 1952. The floral morphology of *Clethra alnifolia* with some notes on *C. acuminata* and *C. arborea*. Bot. Gaz. **113**: 392-413.

KEARNEY, T. H. & PEEBLES, R. H. 1951. "Arizona Flora." Univ. Calif. Press, Berkeley.

MATTHEWS, J. R. & KNOX, E. M. 1926. The comparative morphology of the stamen in the Ericaceae. Trans. & Proc. Bot. Soc. Edinburgh **29**: 243-281.

PALSER, B. F. 1951a. Studies of floral morphology in the Ericales. I. Organography and vascular anatomy in the Andromedeae. Bot. Gaz. **112**: 447-485.

— 1951b. Early endosperm development and embryogeny in *Cassiope hypnoides*. Trans. Illinois Acad. Sci. **44**: 51-57.

— 1952. Studies of floral morphology in the Ericales. II. Megasporogenesis and megagametophyte development in the Andromedeae. Bot. Gaz. **114**: 33-52.

PAYER, J. B. 1857. "Traité d'Organogénie comparée de la Fleur." Paris.

PELTRISOT, C. N. 1904. Développement et structure de la graine chez les Ericacées. J. de Bot. **18**: 309-367, 386-402.

REHDER, A. 1927. "Manual of Cultivated Trees and Shrubs." Macmillan, New York.

SAMUELSSON, G. 1913. Studien über die Entwicklungsgeschichte der Blüte einiger Bicornes-Typen. Svensk Bot. Tidskr. **7**: 97-188.

SMALL, J. K. 1914. Ericales. *With* ABRAMS, L. *Uva-ursi*. *In* North American Flora **29**: 1-102.

THE EMBRYOLOGY OF *WOLFFIA*

SATISH C. MAHESHWARI
University of Delhi, Delhi, India

Introduction

The only embryological account on the genus *Wolffia* is a short note by Gupta (1935) on *W. arrhiza* Wimm. My interest in the embryology of the Lemnaceae arose from a study of Lawalrée's (1945) publication entitled " La position systématique des Lemnaceae et leur classification ". He is opposed to the long-held view of an affinity between the Lemnaceae and the Araceae, and considers the former to be allied to the Helobiales.

Wolffia microscopica[1] occurs abundantly at Delhi in the rainy season. Flowering specimens are seen in October, immediately after the rainy season, and several collections were made in 1953. Material was fixed in formalin-acetic-alcohol as well as in Nawaschin's fluid. It was run up in the alcohol-xylol series and embedded in paraffin in the usual way. Sections were cut 5-10 μ thick and stained in Heidenhein's iron-haematoxylin. Fast green in 90 per cent alcohol was used as a counterstain to increase visibility of the cell walls. Whole mounts of dehydrated and cleared specimens were also studied.

1. The material was sent for determination to the Director, Royal Botanic Gardens, Kew; the Botanist, Forest Research Institute, Dehra Dun; and to Father H. Santapau, St. Xavier's College, Bombay. While no definite identification has been made, the plant may provisionally be put under the species *W. microscopica*.

More recently Dr. W. H. Camp, Department of Botany, University of Connecticut, has written as follows : " The majority of specimens are somewhere in the *Wolffia lingulata* series, of which there are several species in the tropics. The group is in a terrible tangle with materials from various places having been differentially christened without proper study. One thing the family has in common is the habit of morphological plasticity in different environmental conditions, and usually quite a different appearance as plants approach the flowering stage."

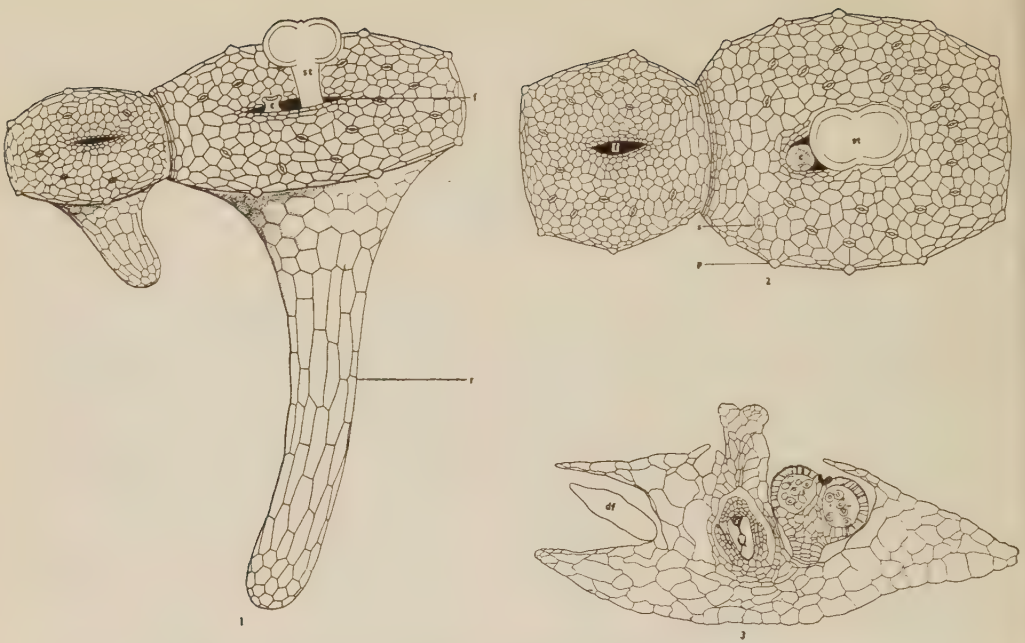
Observations

Flowers — There is a single male and a single female flower, both situated in a common cavity (Figs. 1, 3). The male flower consists of one stamen with a short filament and a large bilobed monothecous anther (Fig. 3). The female flower comprises one carpel with a somewhat broad ovary, short hollow style and an open expanded stigma. There is a single orthotropous basal ovule.

Microsporogenesis and Male Gametophyte — The young anther shows a multicelled hypodermal archesporium consisting of densely cytoplasmic cells (Fig. 4). As it becomes bilobed, a plate of sterile cells divides the archesporium into two groups (Fig. 5). At first the cells of the filament elongate slowly so that the male flower remains enclosed within the cavity of the frond but due to subsequent rapid elongation the anther protrudes beyond the dorsal furrow (Figs. 1, 3).

The peripheral archesporial cells undergo a periclinal division cutting off the primary parietal layer (Fig. 5). The microspore mother cells and their nuclei enlarge rapidly and go through the meiotic divisions (Fig. 7). Meanwhile, the primary parietal layer divides periclinally giving rise to the endothecium and the tapetum layer. As reported by Gupta (1935), a middle layer is absent which is a comparatively rare feature in angiosperms. According to Lawalrée (1952) a middle layer is also absent in *Lemna*. Caldwell's (1899) report of its occurrence appears to be incorrect.

Both the microsporangia of an anther may not develop synchronously. Whereas one of them may show fully formed tetrads, the mother cells in the other may not yet have completed Meiosis II. Frequently there is a complete degeneration



FIGS. 1-3 — Fig. 1. Entire plant with one daughter frond (*c*, carpel; *f*, furrow; *r*, root-like process; *st*, stamen). × 45. Fig. 2. Magnified view of dorsal side of similar plant. × 45. Fig. 3. V.s. mature frond showing one male and one female flower. × 100.

of the contents of one of the pollen sacs.

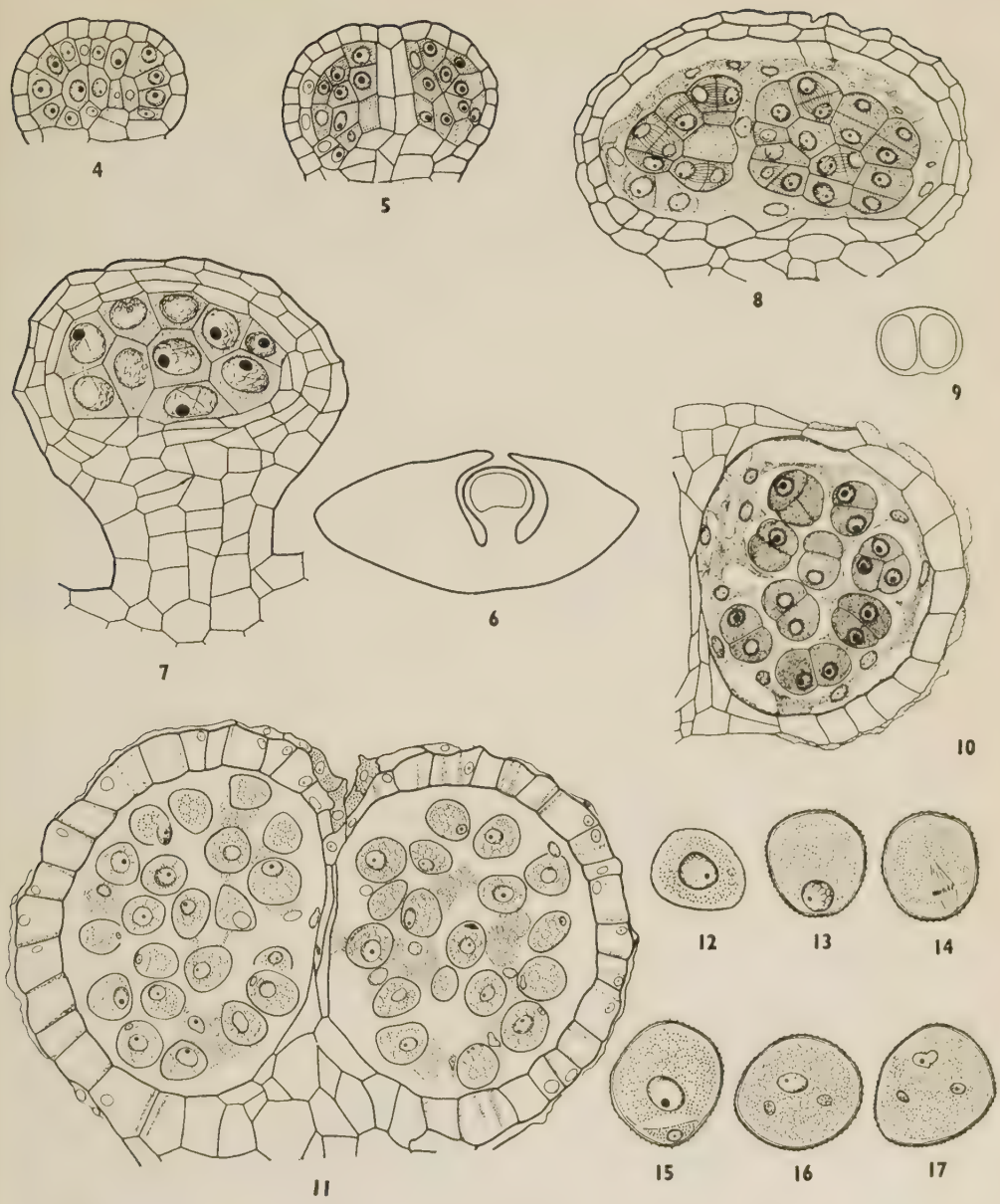
As the anther matures, the epidermal cells become greatly stretched and flattened and almost unrecognizable. The endothecial layer acquires prominent fibrous thickenings.

The tapetal cells remain uninucleate. By the time reduction divisions are over, they lose their inner walls, the protoplasts wander inside the loculus, coalesce and form a true periplasmodium in which are embedded the microspore tetrads (Figs. 8, 10, 11). Gradually, as the pollen grains mature, the periplasmodium is consumed.

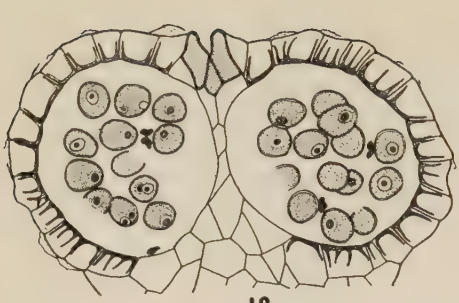
Caldwell (1899) states that in *Lemna* the tapetum is sometimes derived from the sporogenous cells and sometimes from the wall layer. He further points out that many of the mother cells disorganize, and together with the tapetum nourish the remaining mother cells. To quote his own words: " These broken down mother cells frequently form incomplete chains extending into and almost across the loculus, though such masses are usually found near the tapetum. They react to stains as the tapetum, and doubtless assume the function of the latter as nutritive tissue." Caldwell's description and figures seem to indicate, however, that he was dealing with the tapetal periplasmodium and not with any tissue derived from the degenerating mother cells.

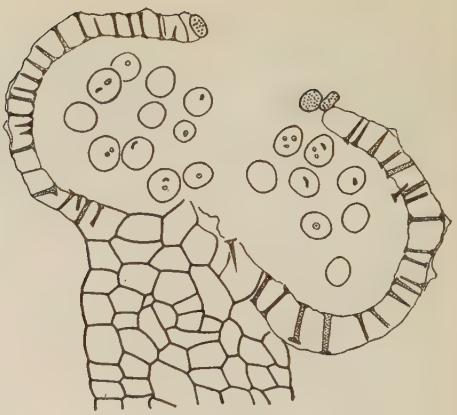
The divisions leading to the formation of the tetrads are successive. Both tetrahedral and isobilateral tetrads occur. The mature pollen grains are 3-celled (see Figs. 12-17 for the developmental stages) and the exine shows spinulose thickenings. Figs. 18-22 illustrate the mode of dehiscence of the anther.

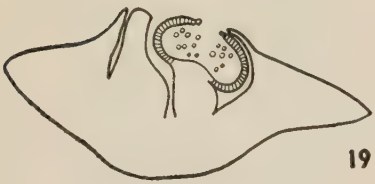
FEMALE FLOWER — The female flower is situated on the anterior side, that is on the side on which the pouch of the frond is located (Figs. 1-3). The ovary stands upright on a cushion of parenchymatous tissue situated on the base of the cavity. When mature, the stigma and style pop out of the dorsal furrow. The carpel is



FIGS. 4-17 — Fig. 4. V.s. young anther showing archesporium. × 504. Fig. 5. Same, older stage, showing cutting off of primary parietal layer. × 504. Fig. 6. Outline sketch of v.s. frond showing embedded anther at microspore mother cell stage. × 98·3. Fig. 7. Anther of the same showing endothecium, tapetal, and microspore mother cells. × 504. Fig. 8. Older stage showing Meiosis I and formation of tapetal plasmodium. × 504. Fig. 9. Outline sketch of transverse section of anther at tetrad stage. × 98·3. Fig. 10. One loculus of same highly magnified. × 504. Fig. 11. V.s. anther at two-celled pollen grain stage showing thickenings in endothecial layer and disappearance of the epidermis. × 504. Fig. 12. Uninucleate microspore. × 1161. Fig. 13. Older stage showing migration of nucleus towards the periphery. × 1161. Figs. 14, 15. Cutting off of generative cell. × 1161. Fig. 16. Three-celled pollen grain. × 1161. Fig. 17. Older stage showing amoeboid shape of vegetative nucleus. × 1161.







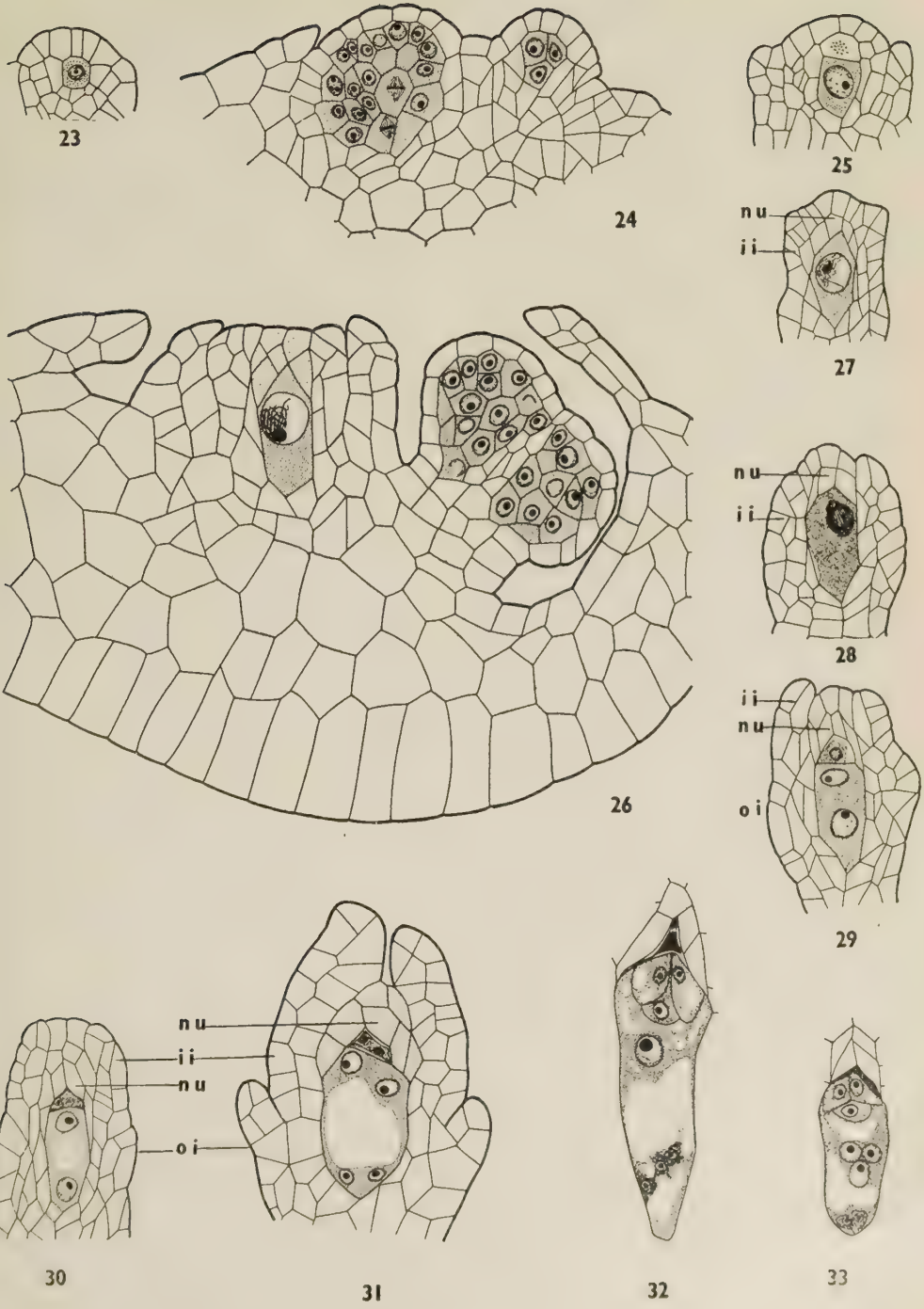
FIGS. 18-22 — Fig. 18. V.s. anther showing degenerated cells above the septum, which aid in dehiscence. × 309. Figs. 19, 20. Outline sketches of plants showing dehisced anthers. × 309. Fig. 21. Anther of Fig. 19 highly magnified. × 309. Fig. 22. Anther of Fig. 20 highly magnified. × 309.

a hollow flask-shaped structure with a style which opens out at the flared and funnel-shaped stigmatic end. It is interesting to note that the ovule is initiated first and the carpellary primordium are seen only at the megaspore mother cell stage (Figs. 24-26).

OVULE — In *Wolffia* the ovule is orthotropous and remains so until maturity. In early stages the nucellus consists of 1 to 2 layers of cells (Fig. 24). It is crushed and consumed, even before the reduction divisions, except for a few cells which persist at the apex of the megaspore mother cell (Figs. 24, 25, 26). Together with the nucellar epidermis they form a cap which can be distinguished even in the mature seed.

→

FIGS. 23-33 — Fig. 23. Portion of l.s. ovule showing hypodermal cell. × 559. Fig. 24. Portion of v.s. frond showing young anther at left and ovule at right. The latter shows a three-celled archesporium. × 559. Fig. 25. Division of archesporial cell to form the primary parietal cell and the megaspore mother cell. × 559. Fig. 26. Portion of v.s. frond showing male and female flowers. The ovule is at the megaspore mother cell stage. × 559. Fig. 27. Megaspore mother cell; on the sides the nucellus has already degenerated. × 559. Fig. 28. Telophase of Meiosis I. × 559. Fig. 29. Two-nucleate embryo sac with degenerating upper dyad cell. × 559. Fig. 30. Same, here the nucleus of the upper dyad cell has also divided. × 559. Fig. 31. Four-nucleate embryo sac. × 559. Fig. 32. Mature embryo sac. × 559. Fig. 33. Abnormal embryo sac showing three polar and two antipodal nuclei. × 559.



FIGS. 23-33.

The two-layered inner integument appears simultaneously with the nucellus and projects slightly beyond the latter (Fig. 24). The outer integument is initiated at a much later stage and remains shorter than the inner one. Figs. 29 and 30 show ovules in which it has just appeared.

Megasporogenesis and Female Gametophyte — The nucellar primordium appears as a small mound of tissue on the dorsal side of the frond. The archesporium is hypodermal and ordinarily one-celled (Fig. 23). Sometimes, however, there may be more than one cell (Fig. 24). At this stage archesporial cells are also distinguishable in the male flower situated on the left.

The archesporial cell undergoes a periclinal division forming a smaller wall cell and a larger megaspore mother cell (Fig. 25). The former divides both anticlinally and periclinally forming two layers of parietal tissue (Figs. 27-33). The megaspore mother cell enlarges considerably and encroaches on the nucellus so that only a few cells survive at the apex (Figs. 26-28). On reduction division, it produces an upper smaller and a lower larger dyad cell (Figs. 28, 29). The former usually degenerates soon after its nucleus has divided. As a rule, the nuclear division is not followed by a wall. When wall formation occurs, the upper two megaspores may lie side by side or one above the other (Figs. 31, 32).

The lower dyad cell enlarges and gives rise to the two-, four- and eight-nucleate stages (Figs. 29-33). From the micropylar quartet differentiates the egg apparatus and the upper polar nucleus while the chalazal quartet forms the three antipodal cells and the lower polar nucleus. The development is, therefore, of the Allium type.

Fig. 32 represents an organized embryo sac where the polar nuclei have already fused and the antipodals are showing signs of degeneration.

Pollination — The female flower matures earlier than the male. Table 1 will serve to show their comparative development.

TABLE 1

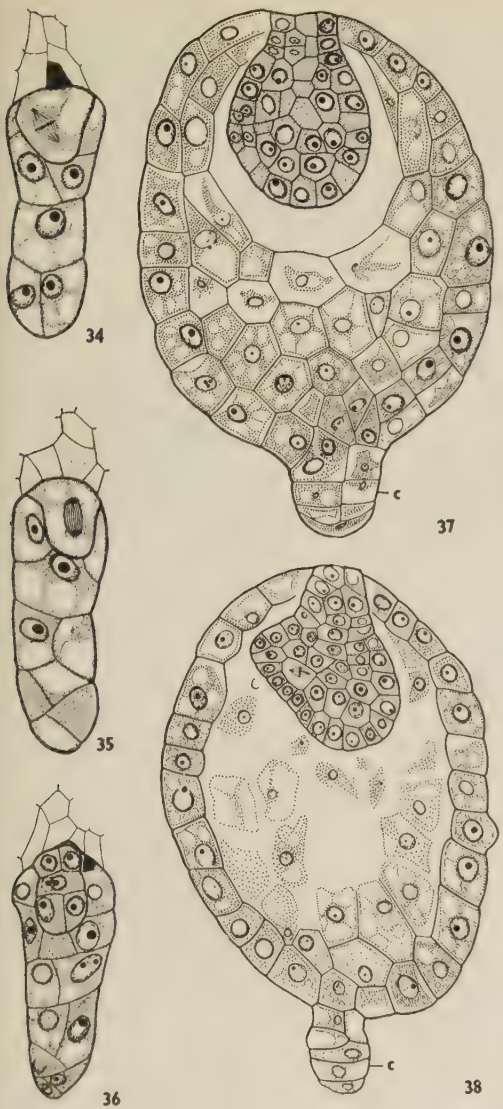
Female flower	Male flower
Young megaspore mother cell with primary parietal cell	Differentiation of primary archesporial cells
Advanced megaspore mother cell	do
Same in synizesis	Primary parietal layer and sporogenous tissue
Meiosis I	Differentiation of endothecial and tapetal layers
Two-nucleate embryo sac	Microspore mother cells
Four-nucleate embryo sac	Prophase of Meiosis I
Eight-nucleate embryo sac	Dyads and tetrads
Fertilized embryo sac	Two-celled pollen grains
Zygote undivided, endosperm 5-6 celled	Three-celled pollen grains

McCann (1942) states that in *Lemna* one of the two stamens matures a little earlier than the carpel, but in *Wolffia*, as also in *Wolffiella* (Mason, 1938), it is the ovary which matures first.

Pollination is effected probably through the agency of rain and wind. The pollen grains germinate on the stigma and the pollen tube grows along the wall of the hollow stylar canal. Fertilization was not observed.

Endosperm — The endosperm is already cellular when the zygote divides. Figs. 34, 35 show 7 and 9 endosperm cells while the zygote is in metaphase and telophase respectively. Such an early formation of walls seems to exclude the probability of a Nuclear type of endosperm.

In *Lemna minor*, Lawalrée (1952) reports a Helobial endosperm. He writes: " La première division du noyau triploïde se fait dans le bas du sac embryonnaire, avec fuseau longitudinal, et est suivie d'une ' séparation ' transversale entre une grande chambre micropylaire, représentant la presque totalité du sac embryonnaire, et une petite chambre chalazale constituée de la partie étroite du sac, immédiatement au-dessus des antipodes dégénérées." His figures do not prove it, however, and some of his photomicrographs seem to suggest that here too the endosperm is Cellular.



FIGS. 34-38 — Figs. 34, 35. Division of the zygote; metaphase and telophase stages. × 875. Fig. 36. Embryo sac with six-celled proembryo and cellular endosperm. × 552. Fig. 37. Globular embryo; endosperm shows a caecum *c* at the chalazal end. × 552. Fig. 38. Older stage; embryo showing differentiation of stem apex. × 552.

Although the division of the primary endosperm nucleus was not observed by me, later stages indicate that the first wall is probably transverse, separating the embryo sac into a larger micropylar and a smaller chalazal chamber. None of the preparations of *Wolffia* showed free nuclei in the micropylar chamber. The pattern of cells in Figs. 34-36 seems to indicate that all nuclear divisions had been followed by wall formation.

Whereas the upper part of the embryo sac broadens a good deal, the basal forms a small pouch or caecum (Figs. 37, 38, 40). This is cylindrical in shape and penetrates deep into the chalaza consuming the cells in its way. Its behaviour is suggestive of its haustorial nature.

The endosperm cells are large and vacuolated, and contain abundant starch. With the development of the embryo, the adjacent endosperm cells are consumed and only the epidermal layer persists in the seed (Fig. 40).

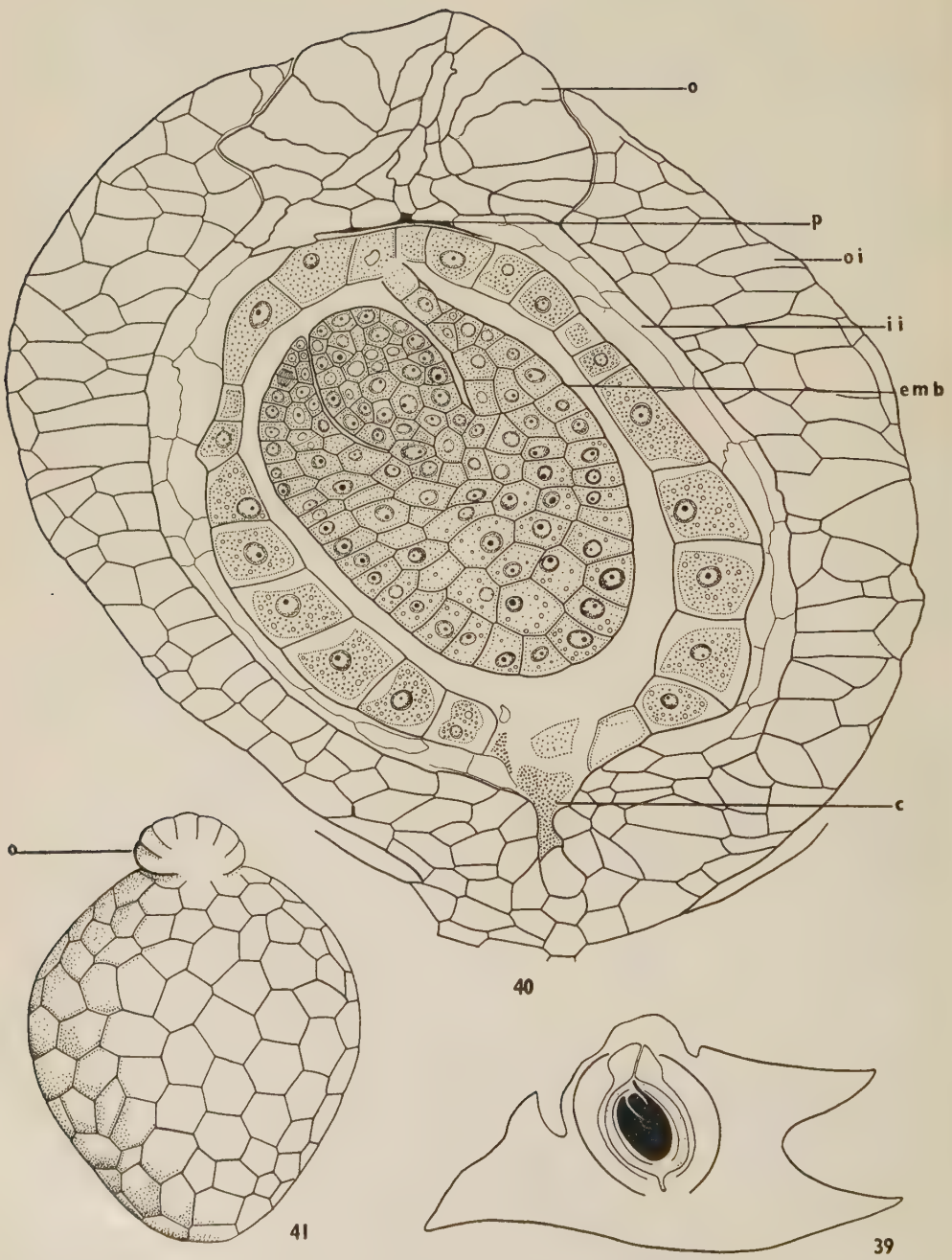
Occasionally, in some cases, the endosperm may develop but the embryo aborts.

EMBRYO — The embryo shows some important departures from the usual sequence of development known in other angiosperms.

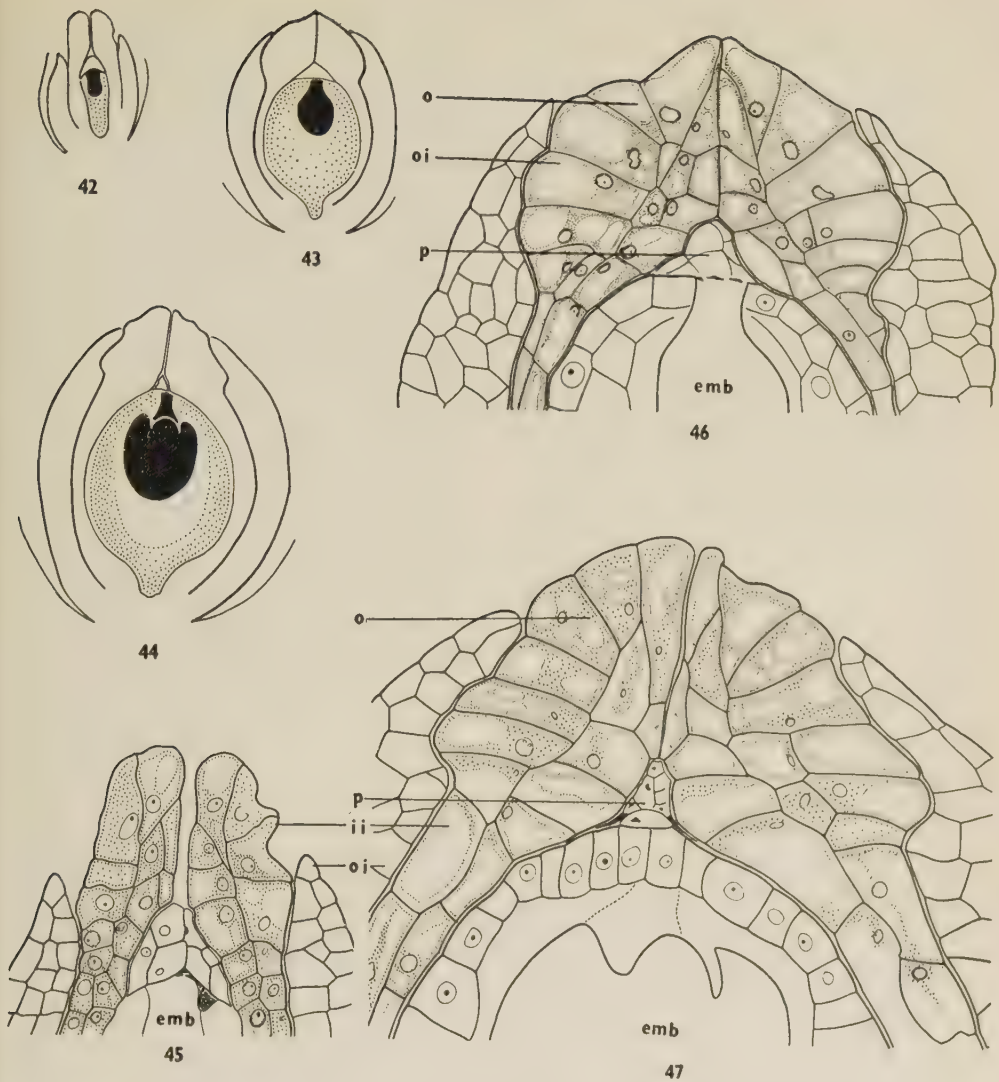
The first division of the zygote is transverse. Fig. 36 shows a three-tiered proembryo with two juxtaposed terminal cells. At the globular stage it has a short suspensor but lacks the histogenic differentiation into dermatogen, periblem and plerome (Fig. 37).

Further development is irregular and leads to the formation of a lateral outgrowth referred to as the ' stem tip ' (Fig. 38). At this time the embryo is about one and a half times as long as broad and more or less pyriform in shape. There is practically no difference in the staining reactions of the cells composing the suspensor and the embryonal mass. Further growth, which is more pronounced in the apical region, displaces the stem tip which comes to lie near the suspensor. While this shift is taking place, the adjacent embryonal tissue extends beyond the stem tip and forms a collar around it.

The mature embryo consists of a short suspensor, a massive cotyledon and the stem tip. There seems to be no indication of a radicle (Fig. 40). The cells of the stem tip are smaller, more compact, and richly cytoplasmic, while the rest of the embryo consists of large vacuolated cells.



FIGS. 39-41 (*c*, caecum; *emb*, embryo; *ii*, inner integument; *o*, operculum; *oi*, outer integument; *p*, nucellar cap) — Fig. 39. Outline sketch of l.s. frond containing a mature seed. × 75. Fig. 40. Magnified view of v.s. seed. × 527. Fig. 41. External view of a mature seed. × 201.



FIGS. 42-47 (*emb*, embryo; *ii*, inner integument; *o*, operculum; *oi*, outer integument; *p*, nucellar cap) — Figs. 42-44. Outline drawings of seeds at various stages of development. × 94. Fig. 45. Enlargement of Fig. 42, showing the inner and outer integuments, micropyle and nucellar cap. × 473. Figs. 46, 47. Older stages showing closure of micropyle and crushing of the nucellar cap. × 473.

FRUIT AND SEED — The mature seed remains enclosed within the collapsed carpellary tissue. As the latter ruptures, it escapes through the dorsal furrow.

The seed coat consists of a stout and thick testa and a thin membranous tegmen. Although initially two-layered in the seed, the outer integument becomes three to four cells thick (Fig. 40). The inner integument is also two-layered except at the apex (Fig. 45).

A characteristic feature is the formation of an operculum which appears like a stopper at the micropylar end of the

seed and was first recorded by Hegelmaier (1868). It originates from the apical cells of the inner integument which show conspicuous enlargement even before fertilization. During the development of the embryo, cells of both the layers elongate and divide anticlinally (Figs. 45-47). They also become lignified and take a red stain with Safranin-Fast Green.

The outer integument fits close to the operculum and from outside the seed gives a more or less streamlined appearance (Fig. 41).

As has been mentioned earlier, except at the apex the nucellus degenerates even before megasporogenesis. The persisting cells form a nucellar cap which is completely covered by the operculum. The disorganized remains of the nucellar cap may be seen even in the mature seed (Fig. 40).

Discussion

The family Lemnaceae is considered by most botanists to be allied to the family Araceae, and it is thought that the Lemnaceae have arisen as a result of regressive evolution somewhat in the following sequence (see Lawrence, 1951):

Araceae (*Pistia*) — *Spirodela* — *Lemna Wolffia*.

According to Lawalrée (1945) the family Lemnaceae does not show a close affinity with the Araceae, but has been derived from the Helobiales. My studies on *Wolffia* do not support this view.

The embryo sac in the Lemnaceae is bisporic, endosperm seems to be cellular with a short chalazal haustorium, and the early divisions in embryogeny are irregular. None of these features are shared by the Helobiales. In all the families of this order, except the Alismaceae and the Butomaceae, the embryo sac is monosporic. Even in the Butomaceae, *Butomus umbellatus* is an exception and has a monosporic embryo sac (Holmgren, 1913; Roper, 1952). The endosperm in the Helobiales is Helobial or Nuclear. The first division of the zygote results in the formation of a terminal cell and a basal cell of which the latter remains undivided and becomes hypertrophied.

On the other hand, in both Lemnaceae and Araceae the endosperm is cellular with a short chalazal endosperm caecum and the mode of embryo development is similar.

Apparently, therefore, the previous assignment of the Lemnaceae to the Spathiflorae along with the Araceae appears to be more satisfactory. However, the systematic position of the Lemnaceae cannot be fully considered unless different members of this and other families under the Spathiflorae have been more thoroughly studied.

Summary

The plant bears two flowers, one male and the other female. The female flower is always located towards the pouch end of the frond and consists of a pistil with a hollow style and a single orthotropous ovule. The male flower consists of a stamen with a bilocular anther. There is a prominent endothecium, but no middle layer. The tapetum is of the amoeboid type and forms a periplasmodium. The division of the microspore mother cells is successive. The mature pollen grains are three-nucleate.

The ovule is crassinucellate and bitegmic. The outer integument does not make its appearance until the embryo sac is two-nucleate. The development is bisporic and the mature embryo sac is eight-nucleate.

The nucellus disappears completely except a few cells at the top which constitute the so-called "nucellar cap" whose remnants can be seen even in mature seed.

The operculum is formed by the inner integument only. The outer integument remains shorter than the inner.

The endosperm is cellular. The first division is transverse. The greater part of the endosperm is derived from the upper cell, while the lower develops into a haustorial outgrowth or "caecum".

The embryo is monocotyledonous and has a short suspensor. The developing embryo lacks the usual histogenic differentiation into dermatogen, periblem and plerome. There is no radicle.

The family Lemnaceae shows greater resemblances to the Araceae than to any family of the order Helobiales.

It gives me great pleasure to express my deep gratitude to Dr. B. M. Johri and Prof. P. Maheshwari, who kindly guided this work.

I am also very grateful to Prof. B. L. Gupta, St. Johns College, Agra, who kindly passed on to me all his materials, slides and a few sketches prepared by him several years ago. Fig. 28 in this paper was kindly supplied by him.

Literature Cited

CALDWELL, O. W. 1899. On the life-history of *Lemna minor*. Bot. Gaz. **27**: 37-66.

GUPTA, B. L. 1935. Studies on the development of the pollen grain and embryo sac in *Wolffia arrhiza*. Curr. Sci. **4**: 104.

HEGELMAIER, F. 1868. "Die Lemnaceen, eine monographische Untersuchung." Leipzig.

HOLMGREN, J. 1913. Zur Entwicklungsgeschichte von *Butomus umbellatus* L. Svensk bot. Tidskr. **7**: 58-77.

LAWALRÉE, A. 1945. La position systématique des Lemnaceae et leur classification. Bull. Soc. Roy. Bot. Belgium **71**: 27-38.

— 1952. L'embryologie des Lemnaceae. Observations sur *Lemna minor*. La Cellule **54**: 305-326.

LAWRENCE, G. H. M. 1951. "Taxonomy of Vascular Plants." New York.

MASON, H. L. 1938. The flowering of *Wolffiella lingulata* (Hegelm.) Hegelm. Madroño **4**: 241-251.

MCCANN, C. 1942. Observations on Indian duckweeds, Lemnaceae. J. Bombay Nat. Hist. Soc. **43**: 148-162.

ROPER, R. B. 1952. The embryo sac of *Butomus umbellatus* L. Phytomorphology **2**: 61-74.

STUDIES IN INDIAN METZGERINEAE—I. *FOSSOMBRONIA HIMALAYENSIS* KASH.*

S. K. PANDÉ[1], T. S. MAHABALÉ[2], Y. B. RAJÉ[3] & K. P. SRIVASTAVA[4]

University of Lucknow, Lucknow, India

Introduction

The Metzgerineae constitute one of the three sub-orders of the Jungermanniales (Evans, 1939) and include 21 genera distributed in eight families as given below:

I. Treubiaceae
  1. *Treubia* Goebel

II. Fossombroniaceae
  2. *Fossombronia* Raddi
  3. *Simodon* Lindb.
  4. *Petalophyllum* Gottsche
  5. *Sewardiella* Kashyap

III. Pelliaceae
  6. *Androcryphia* Nees
  7. *Calycularia* Mitt.
  8. *Pellia* Raddi

IV. Blasiaceae
  9. *Blasia* (Mich.) L.
  10. *Cavicularia* Steph.

V. Pallaviciniaceae
  11. *Pallavicinia* S. F. Gray
  12. *Podomitrium* Mitt.

*Contribution from the Botany Department, Lucknow University, New Series, No. 10

1 and 4. University of Lucknow. 2. University of Poona. 3. S. P. College, Poona.

13. *Symphyogyna* Nees and Mont.
14. *Moerckia* Gottsche
15. *Makinoa* Miyake
16. *Makednothallus* Verdoorn
17. *Hymenophytum* Dumort.

VI. Metzgeriaceae
18. *Metzgeria* Raddi

VII. Riccardiaceae
19. *Riccardia* S. F. Gray
20. *Cryptothallus* Malmborg

VIII. Monocleaceae
21. *Monoclea* Hook.

The systematic position of *Monoclea* is still very much disputed. Some authors include it in the Marchantiales while others assign it to the Jungermanniales. Very recently the question has been critically discussed by Proskauer (1951, p. 265) according to whom "it may belong to the Dumortieroid line" of the Marchantiales.

In India the Metzgerineae are represented by ten genera distributed in different parts of the country: *Fossombronia, Sewardiella, Petalophyllum, Calycularia, Pellia, Blasia, Pallavicinia, Moerckia, Metzgeria* and *Riccardia.*

The genus *Fossombronia* is represented in our flora by two species: *F. indica* St. and *F. himalayensis* Kash. (*F. levieri* St.). The former is confined to South India, while the latter is widely distributed in the Himalayas (5,000-7,000 ft.), Madhya Pradesh (Pachmarhi) and South India. *Petalophyllum indicum* Kash., the only known Indian species of the genus, is restricted to the banks of the rivers Ravi and Beas in the Punjab. *Pellia* is frequently found in the temperate Himalayas. One of its species, *P. calycina* (Tayl.) Nees, which according to Krajina and Brayshaw (1951, p. 59) is a synonym of *P. endivaefolia* (Dicks.) Dum., is fairly common in the outer Himalayas (5,000-8,000 ft.), while, another species, *P. epiphylla* (L.) Lindb., is frequently met with in the neighbourhood of Darjeeling and Sikkim in the Eastern Himalayas, but is less frequently met with in the Western Himalayas. The third species, *P. neesiana* (G.) Limp., appears to be rather rare in India. *Blasia pusilla* (Mich.) L., the only known species of the genus, occurs at several places (6,000-10,000 ft.) in the Western Himalayas. It was collected by Pandé from the neighbourhood of Jamnotri (9,000 ft.), Dwali (9,000 ft.), and Phurkia (10,700 ft.) near Pindari glaciers. *Calycularia* is represented in the Indian flora by three species. One of these, *C. crispula* Mitt., is fairly common in the Himalayas at Mussoorie (7,000 ft.), Gangnani (6,000 ft.), Dhakuri (9,000 ft.), Darjeeling (7,000-8,000 ft.) and Jorpokhari (7,000 ft.). The second species, *C. birmensis* St., has so far been recorded only from Darjeeling in the Eastern Himalayas and Burma. The third species, *C. compacta,* was instituted by Kashyap (1929) for a specimen collected by him from Lahul but, as Kashyap himself admitted, it may be only a form of *C. crispula. Sewardiella,* with its single species, *S. tuberifera* Kash., is exclusively an Indian genus, confined to the Western Himalayas. The genus *Pallavicinia* is represented in the Indian flora by about half a dozen species distributed mostly over the Eastern Himalayas, Assam and South India (Stephani, 1900, 1917; Ghosh & Chakravarti, 1943; Pandé & Srivastava, 1953). *Metzgeria* is known from India by more than half a dozen species occurring in the Himalayas, Khasia and Jayantia Hills and the Western Ghats (Stephani, 1900, 1917; Kashyap, 1929; Chopra, 1938, 1938a). *Riccardia* is richly represented in India. Several of its species are included in a collection of the East Himalayan Hepatics by Decoly and Schaul from the neighbourhood of Darjeeling in the Sikkim Himalayas.

Morphological investigations have been carried out on the various genera of the Metzgerineae and references to literature are available in the contributions of Farmer (1895), Cavers (1910), Campbell (1928), Haupt (1920, 1929), Kashyap and Pandé (1922) and others. The best investigated genera are *Blasia, Pellia, Riccardia* and *Fossombronia.* Of the remaining *Mackinoa* has been studied by Schiffner (1901) and Horikawa (1928); *Calycularia* by Schiffner (1901a), Stephani (1901), Campbell (1913) and Pandé and Udar (1953); *Treubia* by Goebel (1891), Stephani (1891), Grün (1914) and Campbell (1916); *Pallavicinia* by Campbell and Williams (1914) and Haupt (1918); *Monoclea* by Ruge

(1893), Campbell (1898), Cavers (1904), Schiffner (1913) and Proskauer (1951); *Petalophyllum* by Mehra and Vashisht (1950); and *Sewardiella* by Pandé and Misra (1937), and Mehra and Khanna (1950). However, many of the Indian species of this group have not so far been fully investigated. Some of these have now been more or less thoroughly worked out and in this and subsequent articles an effort will be made to present the results of these investigations. The present contribution deals with *Fossombronia himalayensis*.

Historical

Studies on the genus *Fossombronia* go as far back as the 19th century when Leitgeb (1877) made a detailed study of a common European species, *F. pusilla* (L.) Dum. A taxonomic account of 3 Scandinavian species was published by Lindberg (1885). Farmer (1895) gave an account of spore formation in *F. dumortieri* (Hueb. et G.) Lindb. He noted a close correspondence between the nuclear divisions in the sporogenesis of *Fossombronia* and *Pellia*. According to him the haploid number of chromosomes in *F. dumortieri* is 8. Howe's (1899) study of the Hepaticae and Anthocerotes of California includes a description of the common Californian species, *F. longiseta* Aust. Macvicar (1900) gave an account of the common English species *F. cristata* Lindb. Humphrey (1906) made an exhaustive study of the life-history of *F. longiseta*. According to him the development of the antheridium in *Fossombronia* resembles more closely that of *Sphaerocarpus* and *Geothallus* than of any of the higher members of the Jungermannieae. He, therefore, suggested that *F. longiseta* forms a connecting link between such forms as *Sphaerocarpus* and *Riccardia*. Horne (1909) described the discharge of antherozoids in *Fossombronia* and *Haplomitrium hookeri* Nees. According to him in both of these the cells of the antheridial wall undergo progressive changes and absorb water with great avidity. The increase in turgidity breaks the antheridial wall into a number of irregular shreds which sharply curl out thus resulting in a forcible ejection of the antherozoids. Hill (1916) studied *F. crispula*, occurring in the dune region of Indiana, U.S.A. In his account of the Codoniaceae Cavers (1910) regards *Fossombronia* as closely related to *Blasia*, *Noteroclada* and *Treubia* which are thalloid forms of the Anacrogynae showing just a beginning of the formation of genuine leaves like those of the Acrogynae. He also suggests that *Fossombronia* may represent an ancestral form of the foliose hepaticae. Kashyap (1915) dealt with the taxonomy and some features of the morphology of *F. himalayensis*, the species under consideration. It forms perennating tubers at the end of the vegetative season, and its spore sculpture closely resembles that of *F. cristata*. Stephani (1900, 1917) presented a monographic account of this genus. He describes *F. laevieri* St. from the Himalayas which, according to Kashyap (1929), is identical with *F. himalayensis*. Müller (1906-11) has given a detailed taxonomic account of the European species of *Fossombronia*. Schiffner (1909) assigned 26 species to this genus distributed over the globe. Haupt (1920) made a detailed study of the life-history of *F. cristula*, a North American species. Showalter (1927) described the process of the fertilization in *F. angulosa* (Dicks.) Raddi and observed some abnormalities in the organization of the archegonia. According to Heitz (1927), as figured by Höffer (1932, p. 189), the haploid number of chromosomes in *F. caespitiformis* is 9. Chalaud (1929, 1930) studied in detail the complete life cycle of *F. pusilla* Dum., and at the same time Haupt (1929) investigated another American species *F. longiseta*. A noticeable feature of this contribution is the development of the antheridium which differs from the previous account given by Humphrey (1906). According to Haupt (1929) the primary antheridial cell divides by a horizontal wall into an upper cell which produces the body of the antheridium and a lower cell which forms the stalk, the process being essentially the same as in other Jungermanniaceae. The author also reports that this species has 6 neck canal cells and the embryo is of the filamentous type. Mehra (1938)

FIGS. 1-8 — Fig. 1. Plants showing habit. Fig. 2. A few thalli enlarged. Fig. 3. Plant showing arrangement of leaves. Fig. 4. Plant bearing mature sporophyte; *p*, perianth. Fig. 5. Plant with dehisced capsule; *t*, tuber. Figs. 6-8. Plants with perennating tubers; t_1, t_2 and t_3, tubers of three successive years.

studied the chromosome number in some Indian members of the Codoniaceae and found that the diploid number of chromosomes in *Petalophyllum indicum*, *F. himalayensis* and *S. tuberifera* is 18. He also noticed an interesting abnormality in the young sporogonium of *F. himalayensis* in which the archesporial tissue was segregated in two groups by the sterilization of cells in the middle of the young capsule. In two preliminary notes Pandé (1926), and Pandé, Mahabalé, Rajé and Srivastava (1953) described the morphological features of *F. himalayensis*. The details of the life-history are presented here.

Material and Methods

The material for the present study was collected from several localities in the Western Himalayas (Mussoorie, Lansdowne, Naini Tal, Ranikhet); Madhya Pradesh (Pachmarhi); and parts of the Western Ghats (Lonavala, Panchgani, Castle Rock, etc.). Land's and Schaffner's fixatives, chromo-acetic acid and Allen's modification of Bouin's fluid were tried and found successful; the last fixative proved especially useful for cytological studies. The material was washed, dehydrated and stored in equal parts of 70 per cent alcohol and glycerine. It was taken up through the usual grades of alcohol, cedar wood oil and xylol and embedded in paraffin. Sections, generally 4-10 μ thick, were cut and stained with Heidenhain's or Delafield's haematoxylin with or without a counter stain of safranin.

Gametophyte

Habit and Habitat — *F. himalayensis* grows singly or in compact clusters (Fig. 1) among grass, mosses and Cyanophyceae. The thalli, growing singly in exposed places, are comparatively larger (about 8 mm.) than those growing crowded together. The plants are light green in colour, and the thalli growing in clusters, due to the overcrowding of the leaves near the apex, often present a miniature cauliflower-like appearance.

The plant body consists of an elongated axis or midrib, more or less flattened above and convex below with the leaves borne bilaterally (Fig. 9). Very often the plant arises from the tuber of the previous year and the older portion of the thallus remains preserved (Figs. 6-8). The gametophyte may be simple, or, especially in the case of solitary and vigorously growing plants, dichotomously branched. The rhizoids arise from the cells of the lower epidermis of the midrib, and are long, hyaline or violet and unicellular. When growing in a mucilaginous felt of Myxophyceae, the tips of the rhizoids become generally greatly swollen and contorted or even lobed (Fig. 10) as is the case with the rhizoids of some fern prothalli growing in similar situations. Presumably the lobed rhizoids are haustorial. Some of the rhizoids, when injured, harbour fungal filaments (Fig. 11, *h*) which later spread throughout the midrib in the hinder part of the thallus (Figs. 12, 13).

The leaves are alternate and succubous (Fig. 9). They are urn-shaped, pale or light green, thin, translucent and one cell thick, except at the base where they may be two-layered. The cells contain numerous chloroplasts (Figs. 14, 15). The margin of the leaf is wavy and irregularly incised and some of the marginal cells show mucilage secreting papillae (Fig. 15, *m.p.*). A few mucilage hairs (Fig. 16), completely invested by the young leaves, are noticeable near the growing point of the thallus.

F. himalayensis is monoecious (Figs. 9, 17) and protandrous. Rarely it may be dioecious. A large number of antheridia are borne either singly or in groups on the stem near the leaf axils (Figs. 9, 17). In fresh specimens the ripe antheridia are easily detected with the help of a pocket lens because of their reddish colour. The archegonia arise laterally on the midrib in the axils of the leaves near the growing point and occur in small clusters (Fig. 18) but never at the apex itself. Each archegonial group is invested, at maturity, by a bell-shaped perianth (Figs. 50, 51) which may be fused or free on one side. In older plants the apex of the thallus grows down into the soil and forms a tuber (Figs. 5-8) as in *F. tuberifera* (Goebel, 1930), *Aitchisoniella himalayensis* (Kash-

yap, 1914), and *Sewardiella tuberifera* (Kashyap, 1915, 1929).

The Growing Point and Structure of the Thallus — The growing point of the thallus (Figs. 19-21) lies horizontally in the midst of young leaves and mucilage hairs which completely encircle it. The mucilage hairs are short-lived, caducous and comparable to the amphigastria of the Acrogynae and the scales of the Marchantiales. All the tissues of the thallus are derived from a single apical cell (Figs. 19-21) which bulges out in the middle and is lenticular, two-sided, and dolabrate. Sometimes, in vertical sections, its outer face appears somewhat flat giving the impression of a wedge-shaped cell. It divides very regularly giving rise to two lateral sets of segments on the two sides. According to Cavers (1910), each lateral segment of the apical cell of *Fossombronia* divides by two transverse walls producing 3 horizontal cells of which the upper and lower give rise to the stem, while the middle cell forms the leaf in precisely the same way as in *Blasia*.

The primordia of the leaves are differentiated in the immediate vicinity of the growing point of the thallus and bear in their axils the initial cells of the reproductive organs. Fig. 18 shows a median longitudinal section of the thallus passing through the growing point. The leaves and the reproductive organs arise alternately on the two sides of the thallus towards its dorsal surface and the growing point continues to function indefinitely. This is especially evident in sections of the thallus taken parallel to the dorsal surface of the midrib.

The internal structure of the thallus is very simple. A transverse section passing through the midrib shows that all the cells are parenchymatous but there is a slight difference in the size of the cells. In the posterior part of the thallus, especially in the older thalli, there is often a copious mycorrhiza (Figs. 12, 13, *mrh*). How the fungus enters the thallus has not been determined but, to judge from the hyphae seen in a number of rhizoids, it is highly probable that the infection takes place through them (Fig. 11) as in *Sewardiella tuberifera* (Chalaud, 1932; Mehra & Khanna, 1950). The fungus is septate but no fructifications of any kind have been noticed. It spreads in the lower cells of the midrib and occasionally extends even to the large food storing cells, but is never found in the vigorously growing regions of the thallus. On account of the mucilaginous cell contents and mucilage papillae, the plants have a great capacity for withstanding desiccation and even after a few months of drying they readily resume their form and activity if immersed in water.

Sex Organs — The antheridia and archegonia develop acropetally on the dorsal surface of the thallus. The antheridia develop either singly or in groups of twos or threes, rarely up to six, on the dorsal surface of the stem near the leaf bases, and tend to be aggregated near the anterior end. Each antheridial group is separated from its neighbour by a small scale representing the leaf which subtends it. Sometimes the antheridia are found mixed with the archegonia as in *F. longiseta* (Humphrey, 1906). The antheridia appear first in the season; at Lonavala they appear about a month after the establishment of the young plants in July and continue to develop till the end of October. When fully mature they are orange-coloured, the colour being due to the disintegration of the chloroplasts in the wall cells of the antheridium.

Development of the Antheridium — Each antheridium arises from a superficial cell cut off from a lateral segment of the apical cell in the vicinity of the growing point. The latter is thus free to grow indefinitely. In early stages it is difficult to distinguish the antheridial from the archegonial initial on account of the great similarity of the two. The former is, however, easily distinguished from the surrounding cells by its elongated shape, dense cytoplasm and large chromatic nucleus.

The antheridial initial projects in a papillate manner above the general level of the thallus. A careful examination of Figs. 22-30 would show that the process of antheridial development is precisely the same as in *F. cristula* (Haupt, 1920), *F. pusilla* (Chalaud, 1929) and other members of the Anacrogynae, and not as in

F. longiseta (Humphrey, 1906). Fig. 23 shows a young antheridium in which both the stalk cell and the body cell have divided by longitudinal walls. Figs. 24 and 25 represent vertical longitudinal sections of comparatively older antheridia. The fertile tissue shows two primary spermatogenous cells which later produce a large number of more or less cubical cells lying within the one-layered wall of the antheridium (Fig. 29). The stalk of the mature antheridium consists of four longitudinal rows of cells, 4 or 5 cells high. Occasionally it may be only 2 or 3 cells high. Further development follows the usual course as in the other Jungermanniaceae. The primary spermatogenous cells divide rapidly into many small-sized sperm mother cells. A transverse section of a nearly mature antheridium (Fig. 29) shows innumerable sperm mother cells ready to be converted into spermatids. The mature antheridium is spherical or oval (Fig. 30). The sperm mother cells have highly granular cytoplasm and prominent nuclei and nucleoli. The antheridial wall is one-cell thick and the wall cells do not contain chloroplasts. Generally the cells towards the terminal part of the antheridium are larger than those elsewhere.

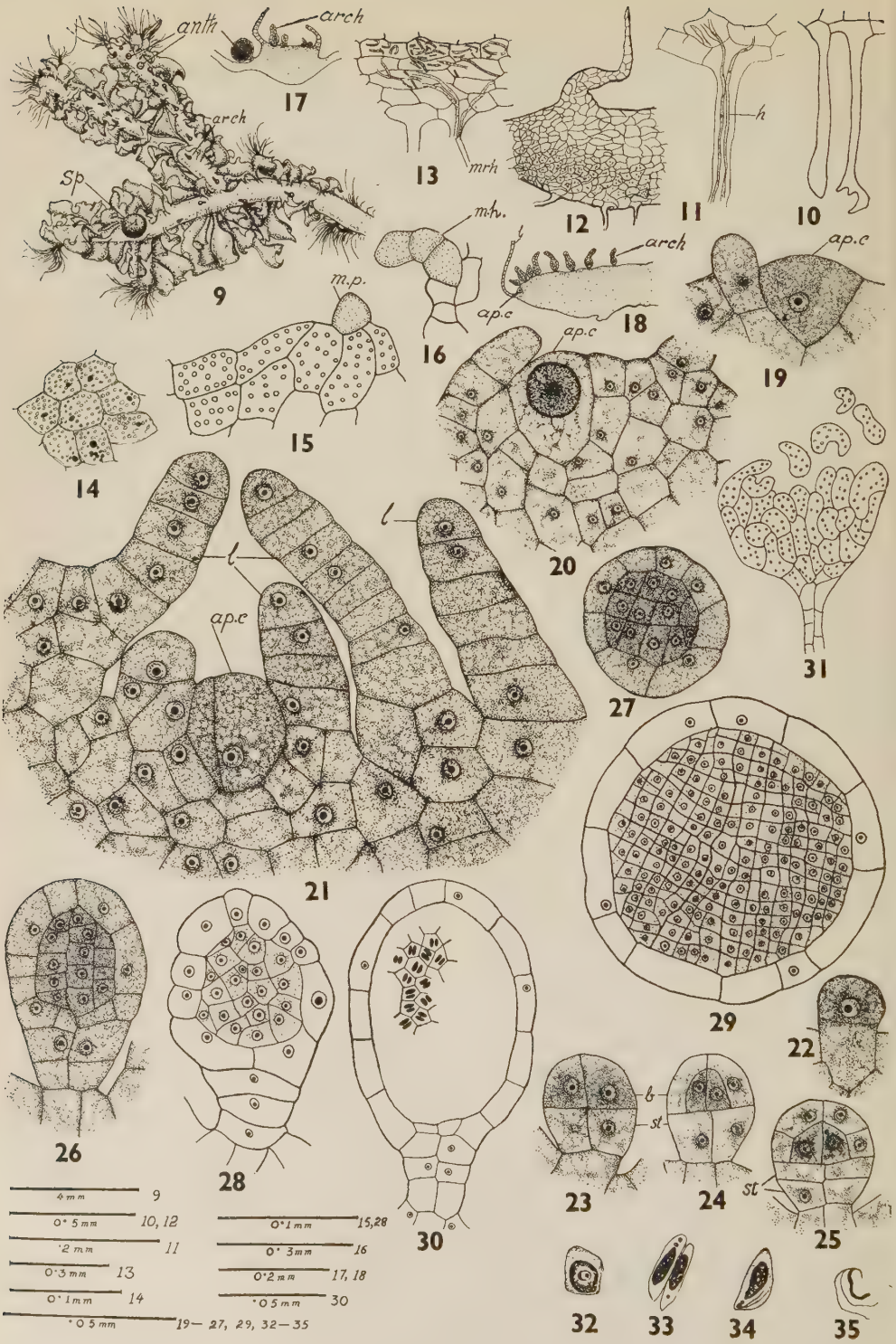
Spermatogenesis — This has been described in some liverworts by Ikeno (1903), Wilson (1911), Woodburn (1911, 1913), Sharp (1920), Yazawa (1931) and others. The number of Jungermanniaceae worked out so far, however, is not large. It has been described in *Blasia* by Woodburn (1913) and Sharp (1920). Yazawa (1931) has studied it in *Makinoa crispata*, Humphrey (1906) in *F. longiseta* and Chalaud (1929) in *F. pusilla*.

Fig. 32 shows a sperm mother cell of *F. himalayensis* at the resting stage before the final spermatogenous mitosis. Later on each one of the sperm mother cells divides diagonally giving rise to two small wedge-shaped cells separated only by a thin membrane (Fig. 33). The achromatic spindle is placed obliquely and at one end lies the blepharoplast. After the restitution of the nucleus in the two spermatids, the blepharoplasts lie outside the nuclear membrane, in the acute angle formed by the two sides of the spermatids. As the nuclear membrane is being formed the blepharoplast gets slightly elongated. Meanwhile another small chromatic body appears in the cytoplasm and lies very close to the blepharoplast (Figs. 33, 34). This is the so-called "Nebenkern" of Nägeli. Next, the cytoplasm becomes vacuolated and the nucleus gets attenuated. Both the "Nebenkern" and the blepharoplast get attached to the narrow end of the attenuated nucleus and the two cilia begin to develop from them. The nucleus constitutes the head of the antherozoid. The cilia elongate and surround the cytoplasmic vacuole. The conversion of the spermatid into a spermatocyte is now complete and after a short time the spermatozoid escapes from the spermatocyte. The larger cells at the tip of the antheridial wall have mucilaginous contents which absorb water and tear the wall into shreds (Fig. 31) so as to liberate the sperms with great rapidity and force. Sometimes, when the plants were being examined under the dissecting microscope, it was noticed that all the sperms gush out within a few seconds of the rupture of the antheridium. Each sperm (Fig. 35) has a small nuclear portion, which stains deeply with iron haematoxylin. Although the species is monoecious, due to its pronounced protandry, there is a very great probability of cross-fertilization.

The haploid number of chromosomes in *F. himalayensis* as counted in the dividing cells of the antheridium is 9. The same number has been given for *F. caespitiformis* (Heitz, 1927; Lorbeer, 1934, quoted in Mehra, 1938), and *F. angulosa* (Lorbeer, 1934).

Development of the Archegonium — The archegonia generally lie in the immediate vicinity of the growing point (Fig. 18) and are protected by the overarching leaves at the base of which they are formed.

The archegonial initial arises from the dorsal cell of a lateral segment of the apical cell. It has granular cytoplasm and stains more deeply than the surrounding cells. It soon divides by a transverse wall into an outer cell and an inner cell (Figs. 36, 37). The inner cell later divides vertically to form a short stalk (Fig. 40). The outer cell divides by a vertical wall


anth
arch
Sp
9
17
arch
13
mrh
12
h
11
10
m.h.
ap.c
16
arch
18
ap.c
ap.c
19
m.p.
15
14
20
l
l
ap.c
21
27
31
29
22
26
28
30
b
st
23
24
st
25
4 mm 9
0·5 mm 10, 12
·2 mm 11
0·3 mm 13
0·1 mm 14
·05 mm 19–27, 29, 32–35
0·1 mm 15,28
0·3 mm 16
0·2 mm 17, 18
·05 mm 30
32
33
34
35

FIGS. 9-35.

into two cells, one larger and the other smaller (Fig. 38). Two more vertical intersecting walls appear in the larger cell resulting in a four-celled stage. Fig. 39 shows a transverse section of a young archegonium in which only two vertical walls have been formed so far. In a longitudinal section (Fig. 40) only two peripheral and a central cell can be seen. In the central cell a periclinal wall cuts off the cap cell (Fig. 41). Meanwhile the peripheral cells divide by vertical walls into five cells (Fig. 45) which later divide transversely to form the five rows of neck cells. Two vertical intersecting walls appear in the primary cap cell producing four cap cells. The axial cell divides transversely into the venter cell and the primary neck canal cell (Fig. 42). Fig. 43 shows an archegonium in which the neck canal cell has divided once. Later the venter cell divides to produce the egg and the ventral canal cell (Figs. 44, 46). Further development of the archegonium is similar to that in the other Jungermanniaceae. The neck of the mature archegonium consists of five rows, each having 7-8 cells. As the archegonium matures the neck cells elongate and the neck is slightly bent in the direction of the leaf. Meanwhile the cells of the venter also divide and form a two-layered jacket (Figs. 47, 48). Thus *F. himalayensis* agrees in this respect with *F. pusilla* described by Leitgeb (1877, p. 113) and Chalaud (1929). In *F. longiseta* (Humphrey, 1906), the venter is usually only one-layered. The number of neck canal cells is 5-6. The mature archegonium thus consists of a small slightly twisted base, a broad venter and a long neck with 5-6 neck canal cells. Ultimately the cap cells are cast off and the neck canal cells fuse and disintegrate. In several preparations the neck canal was seen to be completely filled with a mucilaginous substance through which the sperms had entered (Fig. 48). In some preparations the neck and the venter showed several sperms (Fig. 49). In one or two instances a cell was noticed below the egg, suggesting an abnormality, like the one described by Showalter (1927) in *F. angulosa*. The pro-nucleus of the sperm was seen to lie side by side with the pro-nucleus of the egg, but the actual act of fertilization was not seen. As soon as some of the archegonia are fertilized, the development of new ones is arrested.

As a result of fertilization the cells of the thallus surrounding the base of the archegonium receive added stimulus and form a simple bell-shaped perianth (Figs. 50, 51).

Sporophyte

Fig. 52 shows the fertilized egg. It divides transversely to form a two-celled embryo (Fig. 53). The lower cell does not merely form an appendage as in *Riccardia* and *Madotheca* Dum., but develops into a bulbous foot, as in *Sewardiella* (Pandé *et al.*, in press). The upper cell undergoes a few transverse divisions, as described by Humphrey (1906) and Showalter (1927). How long this process of transverse divisions continues could not be ascertained. Haupt (1920, Fig. 36) figures a six-celled filamentous embryo in *F. cristula*. We have not secured any similar stage but a comparison of the embryos in Figs. 53-55 of this paper with Haupt's Figs. 34-37 would suggest that both the species follow the same sequence

←

FIGS. 9-35 — Fig. 9. Fertile plant showing arrangement of leaves; *anth*, antheridia; *arch*, archegonia; *sp*, sporophyte. Fig. 10. Rhizoids. Fig. 11. A rhizoid with mycorrhiza; *h*, fungal hypha. Fig. 12. L.s. thallus; *mrh*, mycorrhiza. Fig. 13. A few cells in l.s. thallus. Fig. 14. Leaf cells showing chloroplasts. Fig. 15. Marginal cells of leaf showing chloroplasts and a mucilage papilla (*m.p.*). Fig. 16. Mucilage hair (*m.h.*). Fig. 17. V.s. thallus. Fig. 18. L.s. thallus; *ap.c*, apical cell. Fig. 19. T.s. growing point. Figs. 20-21. Longitudinal horizontal section through growing point. *l*, leaf. Fig. 22. L.s. antheridial initial. Figs. 23-26, 28. L.s. antheridia in different stages of development; *b*, body; *st*, stalk. Fig. 27. T.s. antheridium more or less of the age shown in Fig. 26. Fig. 29. T.s. nearly mature antheridium. Fig. 30. L.s. antheridium with spermatids. Fig. 31. Antheridium which has discharged the sperms. Fig. 32. A spermatogenous cell. Fig. 33. Two spermatids produced from a sperm mother cell. Fig. 34. Spermatid showing blepharoplast and " Nebenkern ". Fig. 35. Spermatozoid,

in early embryogeny. Fig. 55 shows a filamentous embryo in a longitudinal section. It consists of somewhat elongated cells in the apical and basal portion. Two of the middle cells of this embryo have not so far divided longitudinally. An examination of older embryos (Fig. 56) indicates that the divisions in the middle part of the embryo are rather slow, while those in the upper part are rapid, producing ultimately a large apical capsule (Fig. 57). Periclinal divisions take place in this region separating the cells of the capsule wall from the central archesporial cells which are large, rich in cytoplasm and stain deeply (Figs. 57, 58). By successive divisions these give rise to sporogenous cells which later form the spore mother and elater primordial cells (Fig. 59) and completely fill the cavity of the capsule. By this time the wall of the capsule becomes double layered (Fig. 57) as in *Madotheca*. In a mature capsule the wall is three layered at the apical end. The divisions of the archesporial cells are uniform for some time, but later some cells divide more slowly and become somewhat elongated and form the elaters while the other cells give rise to spore tetrads. In early stages the spore mother and the elater primordial cells form a compact tissue, but later the former become separated and more or less spherical (Fig. 60). By further divisions they form the sporocytes which show four distinct furrows (Fig. 61). The cytoplasm of the sporocytes is highly granular and vacuolated. The nucleus, which is rather small, undergoes the usual reduction division resulting in a tetrad of spores. The details of the process could not be seen but the cells destined to be elaters surround the tetrads so closely as to suggest a nutritive function.

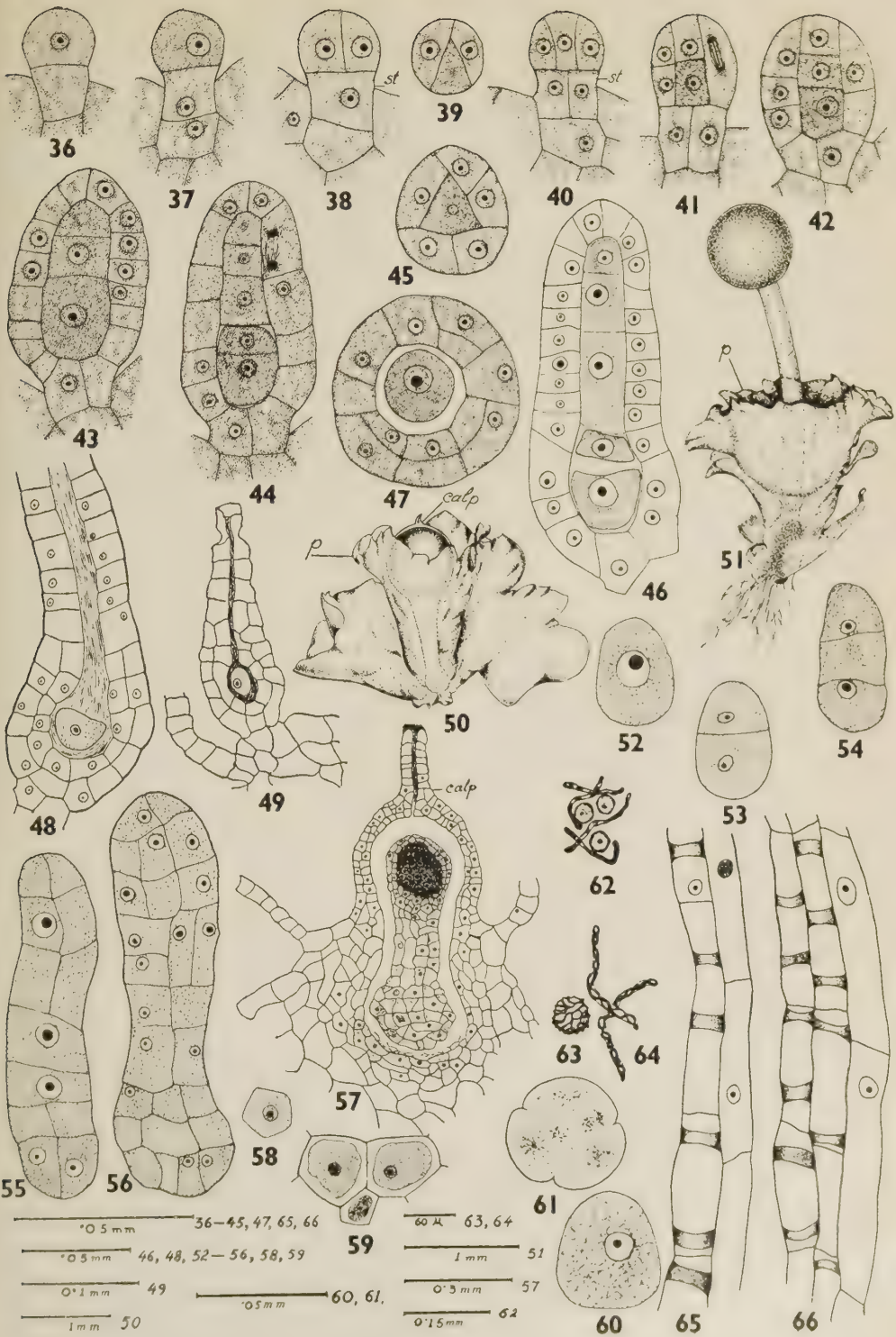
Further development of the spores is similar to that in other genera of the Jungermanniaceae. Many sporocytes apparently disintegrate and probably serve to nourish the remaining spores. The spore tetrads lie in small round cavities in the cytoplasmic mass of the archesporium. Once the spores are mature the cells of the seta grow very rapidly and push the sporogonium out of the perianth. In some cases the seta does not elongate appreciably and the sporogonium remains included in the perianth. The young spores and elaters (Fig. 62) contain chloroplasts and are green in colour, but later these turn deep brown. The spores are 45 μ in diameter and have thick projecting lamellate markings on the exosporium (Fig. 63). The elaters are 140 μ long and have two (sometimes three) spirals (Fig. 64). They are scattered irregularly in the capsule and are thoroughly mixed up with the mature spores. It thus seems that their function is not only to aid dehiscence and dispersal but also to hold the spores together till these are dispersed.

The seta is delicate, cylindrical and composed of parenchymatous cells. It is remarkably long in some plants but short in others.

The hypobasal half of the embryo undergoes repeated transverse and longitudinal divisions to form a bulbous foot which is at first larger than the capsule (Figs. 56, 57). The divisions in its cells are, however, not so regular as in those of the capsule. In the mature sporophyte

⟶

Figs. 36-66 — Fig. 36. L.s. archegonial initial. Figs. 37, 38; 40-44. L.s. archegonia in various stages of development; *st*, stalk. Fig. 39. T.s. young archegonium. Only two peripheral walls have been cut off. Fig. 45. T.s. neck of mature archegonium. Note the five peripheral neck cells enclosing a neck canal cell. Fig. 46. L.s. mature archegonium. Fig. 47. T.s. venter of a mature archegonium showing the formation of two-layered jacket around the egg cell. Fig. 48. Mature archegonium after the disorganization of canal cells. Fig. 49. Archegonium at a later stage than in Fig. 48. Fig. 50. Sporophyte showing calyptra, *calp*; and perianth, *p*. Fig. 51. A sporophyte showing the perianth. Fig. 52. L.s. through oospore. Fig. 53. L.s. two-celled embryo. Fig. 54. L.s. three-celled embryo. Figs. 55, 56. L.s. of embryos in later stages of development. Fig. 57. L.s. young sporophyte enclosed within the calyptra. Fig. 58. An archesporial cell. Fig. 59. Two spore mother cells and an elater primordial cell. Fig. 60. Spore mother cell. Fig. 61. Spore mother cell dividing into spore tetrad. Fig. 62. Immature spores and elaters. Fig. 63. Spore. Fig. 64. Elaters. Figs. 65, 66. Capsule wall in l.s. capsule.


36
37
38
st
39
40
st
41
42
43
44
45
47
46
51
p
48
49
calp
p
50
52
53
54
calp
62
57
63
64
58
61
55
56
59
60
65
66
·05 mm
36–45, 47, 65, 66
·05 mm
46, 48, 52–56, 58, 59
0·1 mm
49
1 mm
50
·05 mm
60, 61,
60 μ
63, 64
1 mm
51
0·3 mm
57
0·15 mm
62

FIGS. 36-66.

the cells of the outermost layer of the foot are more elongated and have denser cytoplasm. Apparently they have a haustorial function. The cells of the thallus underlying the foot are narrow and elongated and become flattened and crushed due to pressure. They stain deeply with safranin.

PERIANTH — The young sporophyte is completely covered by the calyptra which is two or three cells thick (Fig. 57). The cells of the thallus surrounding the fertilized archegonium grow into a distinct organ called the " perianth " (Fig. 50) which grows vigorously for a time, but with the maturation of the spores the seta elongates and the sporogonium projects out of the perianth. The perianth (Figs. 50, 51, *p*) is campanulate, narrow at the base but inflated above with a spreading mouth and wavy margins. Sometimes it is free on one side.

DEHISCENCE OF THE CAPSULE — The cells of the outer layer of the wall are thin and transparent, but those of the next layer show small radial U-shaped thickenings on their inner side (Figs. 65, 66). By their hygroscopic movements and tension, the capsule splits irregularly and the valves may occasionally get further divided into small cell plates. The genus *Fossombronia* differs from *Pellia* and *Riccardia* in that it lacks the elaterophore and the elaters are mixed up with the spores which come out irregularly as the capsule is being torn to pieces. The sporogonium appears in early September and the spores ripen by the end of September-October. When the great majority of the spores are dispersed, the seta falls down so that any of the spores still clinging to the wall of the capsule are thrown off in the immediate vicinity of the plant, or between the leafy lobes. They remain dormant for the whole of the dry season and germinate in about a fortnight after the first showers in June. In the Himalayas and the Western Ghats, *F. himalayensis* completes its life-cycle in about five months.

Vegetative Reproduction

Towards the end of the vegetative season the growing point of the thallus elongates slightly and grows more or less vertically down into the soil (Figs. 5, *t*; 6-8, t_1). It forms a small cellular cushion at the apex and stores starch and other food materials in its cells. It remains in this condition for the rest of the year like the tubers of *Exormotheca tuberifera*, *Riccia dicolor*, *Aitchisoniella himalayensis*, *Sewardiella tuberifera*, *Fossombronia tuberifera*, *Asterella angusta*, etc., resuming growth only in the next vegetative season. The previous year's thallus decays and a new branch sprouts from the resting tuber to give rise to a fresh plant. Sometimes two or three plants may be produced from a single tuber. The tuber may be sessile or borne on a thick cylindrical stalk as reported by Kashyap (1929).

Summary

1. *Fossombronia himalayensis* Kashyap grows commonly in the Western Himalayas, Madhya Pradesh (Pachmarhi), Western Ghats and South India. Our observations on the taxonomy of the species are on the whole in conformity with those of Kashyap.

2. The thallus generally arises from the last year's tuber and is either simple or dichotomously branched. Ventrally it bears violet or hyaline rhizoids arising from the lower epidermal cells.

3. The growing point of the thallus is horizontal. The apical cell is dolabrate and has two cutting faces. It is covered by mucilage hairs and young leaves.

4. The thallus often dichotomises once or twice. The midrib is composed of small compact cells and is mycorrhizal, being occupied ventrally by a septate, intracellular and imperfect fungus.

5. The leaves are lateral succubous and alternate. They are thin, pale green, and one-cell thick all over, except at the base where they are two cells thick, and form a close rosette near the growing point.

6. The species is monoecious and distinctly protandrous. The reproductive organs develop acropetally.

7. The antheridia arise from the lateral segments of the apical cell near the growing point; their development is similar to that in *F. cristula*. The mature antheridium is spherical or oval.

8. The haploid number of chromosomes is 9.

9. The archegonia arise from the lateral segments of the apical cell. The archegonial initial is protected by mucilage hairs and leaves arching over it. The development of the archegonium is similar to that in the other Metzgerineae. The neck consists of five tiers of cells with 7-8 cells in each tier. The venter becomes two-layered before fertilization. The number of neck canal cells is 5-6. Some abnormal archegonia showed cells below the egg.

10. The fertilized egg divides transversely into two cells, of which the hypobasal gives rise to the foot and the epibasal forms the seta and capsule. The young embryo is of the *filamentous* type. In the apical region the capsule wall is three-layered. Elsewhere it is two-layered.

11. The mature sporogonium may project out of the perianth by the elongation of the seta or remain included in it.

12. The spores are lamellate and yellowish brown and mixed with these are found the bi-spiral elaters. Sometimes the elaters may be tri-spiral.

13. The spores mature by the end of September-October. They germinate soon after the advent of monsoon in the following year.

14. Vegetative reproduction by means of apical tubers is very common. The tubers may be sessile or stalked.

The senior author is thankful to the Scientific Research Committee, Uttar Pradesh, for a grant which has greatly facilitated this work.

Literature Cited

Campbell, D. H. 1898. The systematic position of the genus *Monoclea*. Bot. Gaz. **25**: 272-274.

— 1913. The morphology and systematic position of *Calycularia radiculosa* St. Dudley Memorial Volume, Leland Stanford Junior Univ. Publ. Univ. Ser. : 33-61.

— & Williams, F. 1914. A morphological study of some members of the genus *Pallavicinia*. Leland Stanford Junior Univ. Publ. Univ. Ser.: 1-44.

— 1916. The archegonium and sporophyte of *Treubia insignis* Goeb. American J. Bot. **3**: 261-273.

— 1928. "The Structure and Development of Mosses and Ferns." New York.

*Cavers, F. 1904. "Contributions to the Biology of the Hepaticeae. Part I. *Targionia, Reboulia, Preissia, Monoclea.*" Leeds & London.

— 1910. The interrelationships of the Bryophyta. III. Anacrogynous Jungermanniales. New Phytol. **9**: 197-234.

Chalaud, G. 1929. Le cycle évolutif du *Fossombronia pusilla* Dum. Rev. Gén. Bot. **41**: 24-34, 95-105, 129-141, 213-236, 293-306, 353-364, 409-423, 474-497, 541-554, 606-621, 676-699.

— 1930. Le cycle évolutif du *Fossombronia pussilla* Dum. Rev. Gén. Bot. **42**: 31-37, 99-108, 159-163, 219-232, 297-312, 366-384, 429-438, 505-512, 553-588.

Chopra, R. S. 1938. Notes on Indian Hepatics. I. South India. Proc. Indian Acad. Sci. B. **7**: 239-251.

*Not seen in original.

— 1938a. Notes on Indian Hepatics. II. Sikkim-Himalayas and Bengal. Proc. Indian Acad. Sci. B. **8**: 427-439.

Evans, A. W. 1939. The classification of Hepaticae. Bot. Rev. **5**: 49-96.

Farmer, J. B. 1895. On spore formation and nuclear division in the Hepaticae. Ann. Bot. **9**: 469-523.

Ghosh, A. K. & Chakravarty, A. K. 1943. On the occurrence of the genus *Pallavicinia* (thallose anacryogynous Jungermanniales) at Dacca, East Bengal. Sci. & Cult. **8**: 497.

*Goebel, K. 1891. Morphologische und biologische Studien. IV. Über Javanische Lebermoose. 1. *Treubia*. Ann. Jard. bot. Buitenz. **9**: 1-9.

— 1930. "Organographie der Pflanzen." Zweiter Teil. Bryophyten — Pteridophyten. Dritte Auflage. Jena.

Grün, C. 1914. Monographische Studien an *Treubia insignis* Goebel. Flora **106**: 331-392.

Haupt, A. W. 1918. A morphological study of *Pallavicinia lyellii*. Bot. Gaz. **66**: 524-533.

— 1920. Life-history of *Fossombronia cristula*. Bot. Gaz. **69**: 318-331.

— 1929. Studies in Californian Hepaticae. II. *Fossombronia longiseta*. Bot. Gaz. **88**: 103-108.

*Heitz, E. 1927. Über multiple und aberrante Chromosomenzahlen. Abh. naturw. Ver. Hamburg **21**: 48-58.

Hill, E. J. 1916. *Fossombronia crispula* in the dune region of Indiana. Bryologist **19**: 67-68.

*Not seen in original.

HÖFFER, K. 1932. Karyologie. In Verdoorn's "Manual of Bryology". The Hague.
*HORIKAWA, Y. 1928. On *Cavicularia densa* St. Sci. Repts. Tohoku Imp. Univ. Japan, Ser. 4, **3**: 259-264.
HORNE, A. S. 1909. Discharge of antherozoids in *Fossombronia* and *Haplomitrium hookeri*. Ann. Bot. **23**: 159-160.
*HOWE, M. A. 1899. Hepaticae and Anthocerotes of California. Mem. Torrey Bot. Cl. **7**: 1-208.
HUMPHREY, H. B. 1906. The development of *Fossombronia longiseta* Aust. Ann. Bot. **20**: 83-108.
IKENO, S. 1903. Beiträge zur Kenntnis der Pflanzlichen Spermatogenese. Die Spermatogenese von *Marchantia polymorpha*. Beih. bot. Zbl. **15**: 65-88.
KASHYAP, S. R. 1915. Morphological and biological notes on new and little known West Himalayan Liverworts. III. New Phytol. **14**: 1-18.
— 1929. "Liverworts of the Western Himalayas and the Punjab plain." Part I. Lahore.
— & PANDÉ, S. K. 1922. A contribution to the life-history of *Aneura indica* St. J. Indian Bot. Soc. **3**: 79-89.
KRAJINA, V. & BRAYSHAW, T. C. 1951. A new species of *Pellia*. Bryologist **54**: 59-67.
LEITGEB, H. 1877. "Untersuchungen über die Lebermoose." Heft. III. Die frondosen Jungermannieen. Leipzig.
LINDBERG, S. O. 1885. *Scalia hookeri* et Fossombroniae Scandinavicae vivae descriptae. Rev. Bryol. **12**: 33-44.
MACVICAR, S. M. 1900. *Fossombronia cristata*. J. Bot. London **38**: 400.
MEHRA, P. N. 1938. A study of the chromosome number in some Indian members of the family Codoniaceae. Proc. Indian Acad. Sci. B. **8**: 1-7.
— & VASHISHT, B. R. 1950. Embryology of *Petalophyllum indicum* Kash. and a new suggestion of the evolution of the thalloid habit from foliose forms. Bryologist **53**: 89-114.
— & KHANNA, L. P. 1950. Embryology of *Sewardiella tuberifera* Kash. Bot. Gaz. **112**: 31-42.
MÜLLER, K. (1906-11). "Rabenhorst's Kryptogamen-Flora." **6**, Lebermoose (1). Leipzig.
PANDÉ, S. K. 1926. Notes on *Fossombronia himalayensis* Kash. Proc. 13th Indian Sci. Cong. Bombay: 224.
— MAHABALÉ, T. S., RAJÉ, Y. B. & SRIVASTAVA, K. P. 1953. Studies in Indian Metzgerineae. I. *Fossombronia himalayensis* Kash. Proc. 40th Indian Sci. Cong. Lucknow: 84.
— & MISRA, R. N. 1937. On the morphology of *Sewardiella tuberifera* Kash. Proc. 24th Indian Sci. Cong. Hyderabad: 263.
— & SRIVASTAVA, K. P. 1953. The genus *Pallavicinia* Gray in India. 1. *Pallavicinia canarus* St. J. Indian Bot. Soc. **32**: 210-216.
— & UDAR, R. 1953. Studies in Indian Metzgerineae. On *Calycularia crispula* Mitt. Proc. 40th Indian Sci. Cong. Lucknow: 83.
PROSKAUER, J. 1951. Notes on Hepaticae II. The Bryologist **54**: 243-266.
*RUGE, G. 1893. Beiträge zur Kenntnis der Vegetationsorgane der Lebermoose. Flora **77**: 279-312.
*SCHIFFNER, V. 1901. Einige Untersuchungen über die Gattung *Makinoa*. Öst. bot. Z. **51**: 82-89.
*SCHIFFNER, V. 1901a. Neue Untersuchungen über *Calycularia crispula* und *Calycularia birmensis*. Öst. bot. Z. **51**: 285-290.
— 1909. Hepaticeae. In ENGLER & PRANTL'S "Die natürlichen Pflanzenfamilien". I. Teil. 3. Abteilung. Leipzig.
*— 1913. Phylogenetische Studien über die Gattung *Monoclea*. Öst. bot. Z. **63**; 29-33, 75-81, 113-121, 154-159.
SHARP, L. W. 1920. Supermatogenesis in *Blasia*. Bot. Gaz. **69**: 258-268.
SHOWALTER, A. M. 1927. Studies in the cytology of Anacrogynae. III. Fertilization in *Fossombronia angulosa*. Ann. Bot. **41**: 37-46.
*STEPHANI, F. 1891. *Treubia insignis* Goeb. Hedwigia **30**: 190-193.
— 1900. "Species Hepaticarum." **1.** Génève.
*— 1901. Die Elaterenträger von *Calycularia*. Öst. bot. Z. **51**: 256-258.
— 1917-24. "Species Hepaticarum." **6.** Génève.
WILSON, M. 1911. Spermatogenesis in the Bryophyta. Ann. Bot. **25**: 415-457.
WOODBURN, W. L. 1911. Spermatogenesis in certain Hepaticae. Ann. Bot. **25**: 299-313.
— 1913. Spermatogenesis in *Blasia pusilla*. Ann. Bot. **27**: 93-101.
*YAZAWA, H. 1931. On the spermatogenesis in *Makinoa crispata* (St.) Miyake. Cytologia **2**: 157-173.

*Not seen in original.

*Not seen in original.

STUDIES IN POLYPODIACEAE — II. CONTRIBUTIONS TO THE MORPHOLOGY OF *PSEUDODRYNARIA CORONANS* (WALL.) C. CHR.

B. K. NAYAR
Gauhati University, Assam

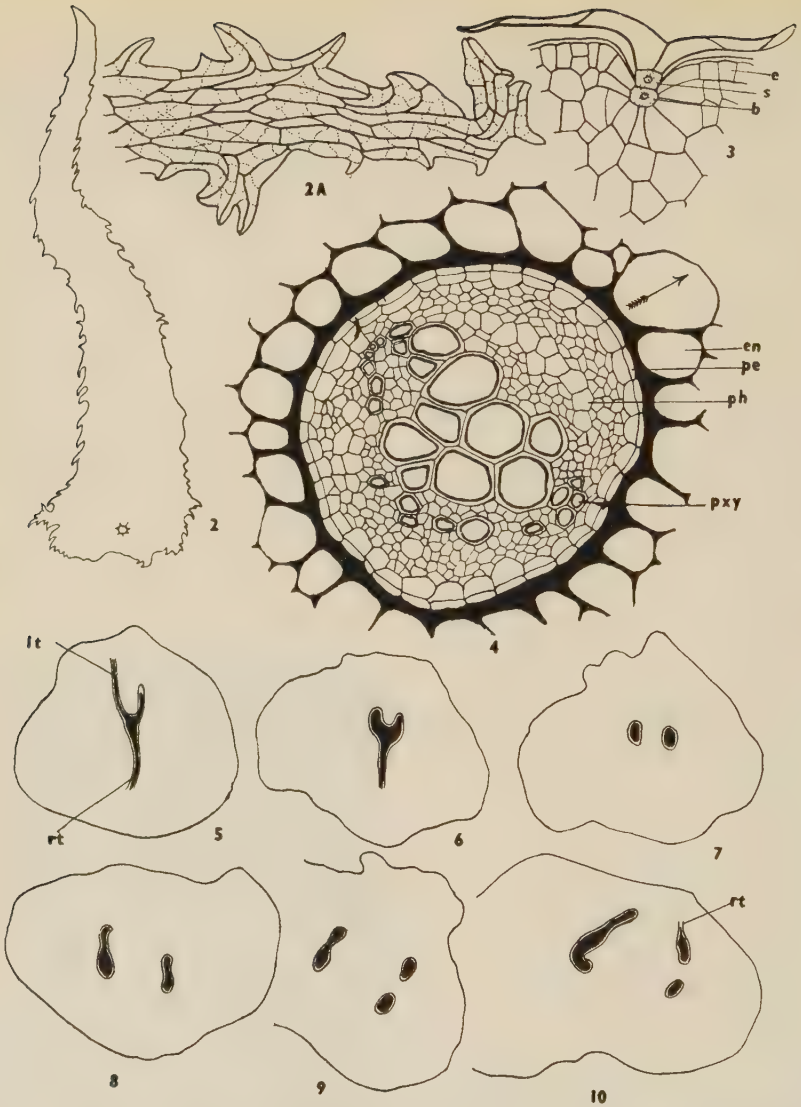
Pseudodrynaria C. Chr., a monotypic tropical fern of majestic appearance and restricted distribution, occurs in N.E. India, Burma, Malaya and Formosa. *P. coronans* (Wall.) C. Chr. is the type and sole species. In tropical forests it grows in shady spots on the trunks and branches of trees, attaching itself to their lateral surfaces, with the leaves borne obliquely with respect to the substratum. Rarely it grows even on shaded rocky surfaces (Fig. 1). It is a large epiphyte with long, stout (up to 7·0 cm. in diameter), fleshy, creeping, sparsely branched rhizome, adpressed to the substratum by a more or less flattened surface. The leaves are large and sessile, with a dilated shallowly lobed base and a pinnatifid upper region, with a strong midrib. The upper portion of the leaf is much wider than the basal and there is a narrow isthmus connecting the two. The roots are highly branched, thin and covered with a dark brown felt of persistent root hairs. They arise from all over the rhizome, either clasping the substratum or ramifying in the humus collected by the bases of the leaves. The rhizome and the basal portions of the midribs of leaves are densely clothed by dark brown lanceolate paleae with dentate margins and recurved tips.

The species was collected at Cherrapunji (Assam, India) in October 1952, and was later grown at Gauhati. Spores were germinated on sterilized canal soil, humus collected from the bases of the leaves of the mother plant, and 3·0 per cent Knop's solution. A study of the mature gametophyte was made mainly from specimens collected from nature.

Rhizome

The rhizome is fleshy, the main bulk being composed of parenchymatous cells with brownish contents and included starch grains. The epidermis is composed of regularly arranged short cells with thickened outer walls in which the continuity is broken here and there by

FIG. 1 Photograph of *Pseudodrynaria coronans* growing on a rocky habitat.



FIGS. 2-10 — Fig. 2. Palea from rhizome. × 16. Fig. 2A. Tip of palea showing intracellular trabeculae (dotted lines). × 60. Fig. 3. T.s. portion of outer cortex of rhizome showing origin of palea (*s*, stalk cell; *b*, basal cell; *e*, epidermis of rhizome). × 160. Fig. 4. One meristele from rhizome; the arrow points in the direction of the centre of the rhizome (*en*, endodermis; *pe*, pericycle; *ph*, phloem; *pxy*, protoxylem). × 260. Figs. 5-10. Serial sections of young rhizome, showing development of dictyostele from protostele and origin of traces to cotyledonary leaves (*lt*, leaf trace; *rt*, root trace). × 16.

small depressions from which the paleae originate. The paleae are one cell thick, peltate, pointing towards the apex of the rhizome and with a stalk composed of two superposed cells attached towards the centre of the broad base (Fig. 2). The cells constituting the paleae are empty, irregular, thin-walled (walls dark brown) and with trabecular connections between the walls (Fig. 2A). The stalk and the basal cell are thin-walled, with dense contents. The latter

is embedded in the cortical parenchyma cells which are arranged in a radiating manner in these regions (Fig. 3). Epidermal cells abutting on the stalk are thin-walled at the region nearest to it. Though not as elaborate as in the related genus *Drynaria* (Nayar & Kachroo, 1953), the structure of the paleae suggests their role in the collection and absorption of water, as in the angiospermous family Bromeliaceae. The vascular system is a gutter-shaped " dictyostele " with the two free margins of the gutter folded along the inner surface and becoming juxtaposed towards the centre on the concave side. Thus the meristeles, in a cross-section, seem to be arranged in a double row bent like a horseshoe, with the concavity facing the dorsal surface of the rhizome (Fig. 22). Each meristele is oval to circular in outline and surrounded by a definite pericycle of small, regularly arranged parenchymatous cells and an endodermis with the inner and radial walls of the cells highly thickened and coloured brownish black (Fig. 4). The xylem consists of pitted tracheids with two or more groups of protoxylem strands, composed of spiral and scalariform tracheids. The phloem surrounds the xylem and is composed of prominent sieve tubes embedded in parenchyma.

Leaf traces are invariably given off from the dorsal surface. The meristeles lying towards the base of the concavity of the stele, where the two infolded margins of the latter meet, are pushed outwards and later become arranged, in a cross-section, in the form of a horseshoe with the concave side facing inwards (Figs. 22, 23, *lt*). These bundles pass through the cortex obliquely and enter the petiole, still preserving their arrangement with the concavity facing towards the apex of the rhizome.

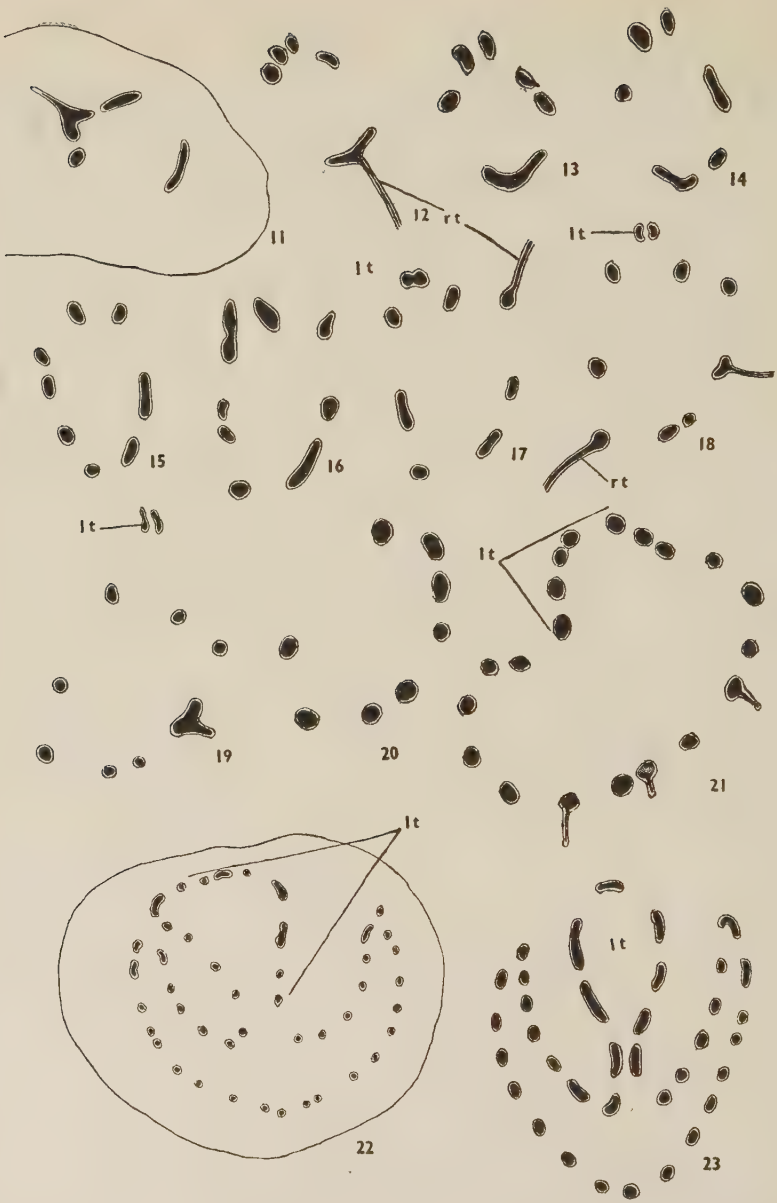
Branching is rare and has no correlation with the leaves. A few meristeles, lying nearest to the surface where the branch is formed, become separated and supply the branch. On entering the branch they are arranged in a circle and as the girth of the branch increases towards its apex, the characteristic configuration of the mature rhizome is attained.

The rhizome in very young stages has a plate-shaped protostele with a root trace and a single leaf trace at diametrically opposite points. Soon a second leaf trace is given off to the next leaf (Fig. 5). The single leaf trace of the cotyledonary leaves preserves its identity in the petiole, branching dichotomously in the lamina. The plate-like protostele soon becomes a gutter-shaped siphonostele, with the gap facing towards the dorsal surface (Fig. 6). It soon splits into two parallel strands (Figs. 7, 8). Later each of these strands disintegrates into a large number of strands arranged in a circle (Figs. 9-15). Root traces are given off irregularly from all the strands. A trace to the third juvenile leaf is given off at this stage from one of the strands lying nearest the dorsal surface. This strand cuts off a single leaf trace from one of its margins (Figs. 16, 17). The trace soon is split into two (Figs. 18, 19), and enters the leaf base, one of the strands splitting further into a larger and a smaller strand, just before reaching the petiole. In the petiole and the midrib the two bigger strands lie towards the adaxial side and give off branches alternately. Towards the tip of the leaf all the three unite forming a single strand.

As the rhizome increases in size, further disintegration of the vascular strands occurs (Fig. 20). Vascular connection to the next juvenile leaf is established by a few of the strands lying towards the upper surface of the rhizome, where the two margins of the disintegrated gutter-shaped stele come together, becoming pushed outwards (Fig. 21), and passing out as in the case of older leaves. By further growth of the rhizome accompanied by the increase in the number of vascular strands, the latter becomes arranged, in t.s., in an ellipse, which is later flattened, leading finally on to the characteristic horseshoe-shaped pattern, where leaf traces regularly originate from the middle of the upper invaginated surface (Figs. 22, 23).

Leaf

Leaves of the mature plant are sessile, pinnatifid, about one metre long and



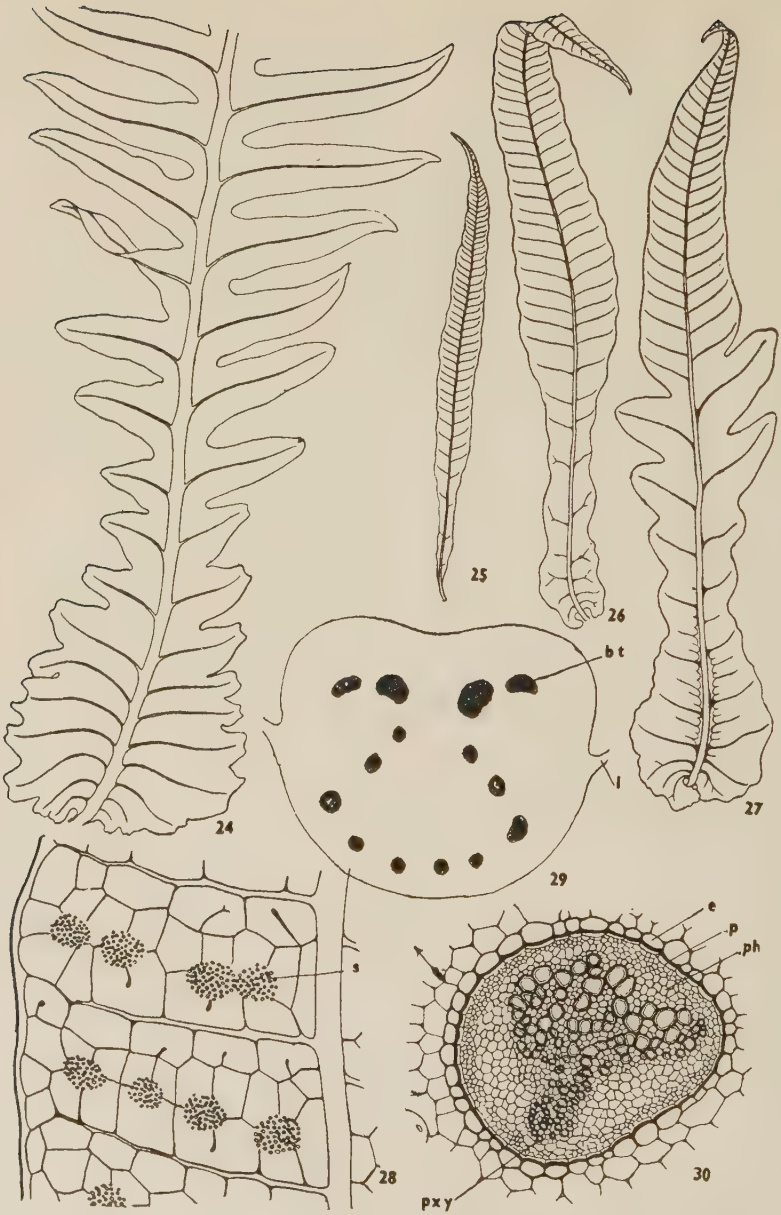
FIGS. 11-23 — Figs. 11-21. Serial transverse sections of the vascular system of young rhizome, showing disintegration of stele (*lt*, leaf trace; *rt*, root trace). × 16. Fig. 22. T.s. mature rhizome showing configuration of stele and the median leaf trace (*lt*). ×$\frac{4}{5}$. Fig. 23. Vascular system of mature rhizome showing outgoing leaf trace (*lt*). × 2.

30·0 cm. broad in the wider regions. They are borne in a single row on the upper surface of the rhizome, usually in a plane oblique to the surface of the substratum. The upper lobes have a broad base and taper gradually towards the tip. The margin of the lower portion of the leaf is only slightly lobed (Fig. 24). When young, the leaf as a whole, and the lobes are circinate. Sparsely arranged

peltate paleae are found on both surfaces of the leaf in the young condition. The leaf lobes are strictly alternate and there is an articulation at the base of each segment, becoming more prominent on drying. On shedding, the leaf segments become detached from the persistent midrib at the articulation, leaving the latter naked. Only the upper photosynthetic segments are shed while the basal region of the leaf is persistent (Fig. 1). The apical segment is usually odd, but is rarely paired due to the apical dichotomy of the midrib as in *Drynaria*. The cotyledonary leaf and the early formed juvenile leaves are simple, entire, lanceolate and petiolate (Fig. 25). Later the characteristic form of the mature leaf is attained by the shortening of the petiole accompanied by broadening of the basal region and lobing of the upper region of the lamina (Figs. 26, 27).

The midrib is very hard, polished, more prominent on the lower surface of the leaf, tapering towards the apex, brownish-green in colour, with the adaxial surface more or less flattened and with a median groove. Branch veins are given off to the side lobes in alternate succession. Minor branches that supply the portion of the lamina lying between two successive lobes (the wings) also arise alternately on either side. The venation of the lamina is reticulate, having free, branched or unbranched vein endings with swollen tips, included in the meshes. The main lateral veins of the segments are connected by fairly regular cross veins and these in turn by usually two tertiary veins parallel to the main lateral veins (Fig. 28). The tertiary veins are almost regularly interconnected between the cross veins. Sori, when present, are found on the tertiary vein nearest the basal main lateral vein, at points where the tertiaries are interconnected. The veins are more prominent on the abaxial surface. Margin of the leaf segments is entire, brownish in older leaves, being composed of thick-walled sclerotic cells. The ground tissue of the midrib is sclerenchymatous with the outer layers having walls much thicker than the inner. The epidermal cells are equally thick-walled. Chloroplasts are present in the outer cortical cells of the young midrib. Vascular bundles are arranged in the form of a horseshoe with its free arms facing the adaxial side (Fig. 29). The bundles lying at the tips of the arms are bigger and give off branch veins to the leaf segments. These branches originate marginally (Fig. 29, *bt*). Each branch is concentric with two or three protoxylem strands towards the outer surface. In structure the vascular bundles of the midrib resemble those of the rhizome (Fig. 30), but the xylem parenchyma is proportionately more in the former. As the midrib tapers towards the upper end of the leaf the number of vascular bundles decreases, the nearer ones becoming merged together, till at the extreme tip there is only a single strand that enters the terminal leaf segment directly.

The leaf lamina is tough and leathery. During development primordia for the lateral segments are laid down at intervals alternately. In mature leaf the epidermis (upper and lower) is composed of large, highly thick-walled cells with simple as well as branched pits on all the walls (Fig. 31). The walls show clear concentric lamellation. All hypodermal cells, like the epidermal cells, are transversely elongated and have thickened walls. A few of them, especially those below the lower epidermis, show lamellated walls with pit connections. In others the wall appears homogeneous, but the outer and the radial walls are thicker than the inner and often show pits. Stomata are restricted to the lower epidermis. The epidermal and the hypodermal cells lack chloroplasts. The mesophyll is undifferentiated and is composed of thin-walled cells with prominent intercellular spaces. Five to six rows of mesophyll cells at the extreme margin of the lamina lack chloroplasts, are very thick-walled with lamellations and pits in the walls and are brownish in colour. Chloroplasts are many and equally distributed in all normal mesophyll cells. At the regions of the veinlets, bands of sclerenchyma connect the thick-walled continuous endodermis of the veinlet to the upper and lower layers of hypodermis. Mesophyll cells abutting on the endodermal cells are devoid of chloroplasts. Structurally the



FIGS. 24-30 — Fig. 24 Basal region of adult leaf. × $\frac{1}{5}$. Fig. 25. Early juvenile leaf with entire margin and petiole. × $\frac{1}{4}$. Figs. 26, 27. Later formed juvenile leaves showing transition to the adult form. × $\frac{1}{4}$. Fig. 28. Portion of lamina of fertile leaf showing venation (*s*, sorus). × $5\frac{1}{2}$. Fig. 29. T.s. midrib of leaf towards base, showing arrangement of vascular bundles (*bt*, trace to side lobes; *l*, portion of lamina). × 8. Fig. 30. One bundle of petiole from the abaxial side; the arrow points to the centre of the petiole (*e*, endodermis; *p*, pericycle; *ph*, phloem; *pxy*, protoxylem). × 160.

veinlets resemble a single vascular strand of the midrib on a reduced scale, but the tracheids are of the spiral type only and the phloem is usually represented by a single layer of cells surrounding the xylem mass.

Root

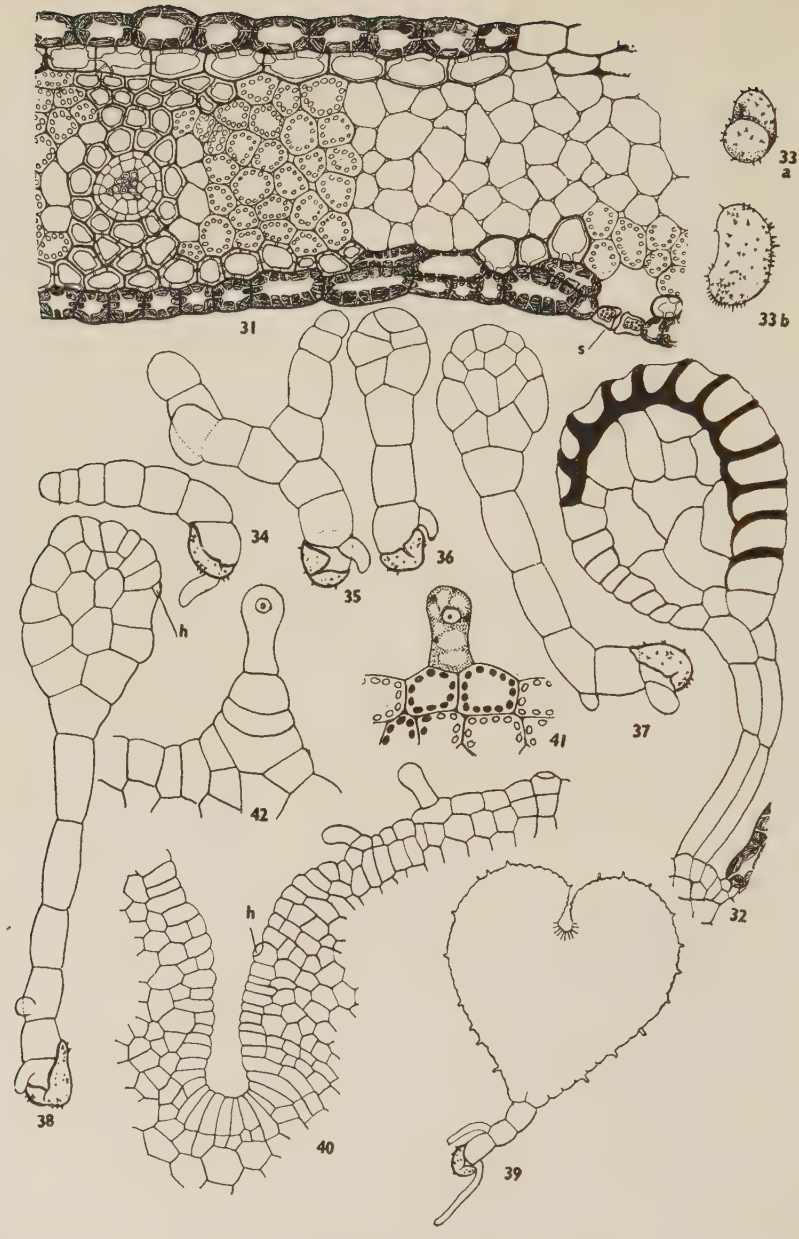
Anatomically the root is similar to that of other Leptosporangiates. There is a superficial piliferous layer bearing long, persistent, brown-walled root hairs and a thick sclerenchymatous cortex. The central stele is small with peripheral protoxylem strands alternating with phloem masses.

Sporogenesis

Sori are restricted to the abaxial surface and are formed all over the leaf, including the broadened base. They are non-indusiate, ovoid in outline, of the mixed type and are borne in transverse rows between the lateral veins on the intersections of the veinlets of the lamina (never on free vein endings)(Fig. 28). Sori arising very close together, especially those borne towards the basal regions of the leaf segments, may merge rarely into each other forming confluent sori. The sorus has a prominent cushion-shaped placentum formed of palisade-like cells. Intermingled with the sporangia are short, club-shaped, thin-walled paraphyses each with a hyaline stalk cell and a brownish terminal cell with thick contents. It has a long stalk, 2 cells thick and usually 4 cells long, a vertical annulus 12 to 14 cells long and a stomium of 4 to 6 cells (Fig. 32). Usually the spore output is 32 per sporangium. The mature spores are slightly yellowish, hyaline, bilateral and bean-shaped with a prominent longitudinal scar on the concave surface (Fig. 33 a, b). The exine is slightly thickened, with minute sparsely distributed protuberances. The intine is very thin. The spores are generally $48 \times 30\ \mu$ in size.

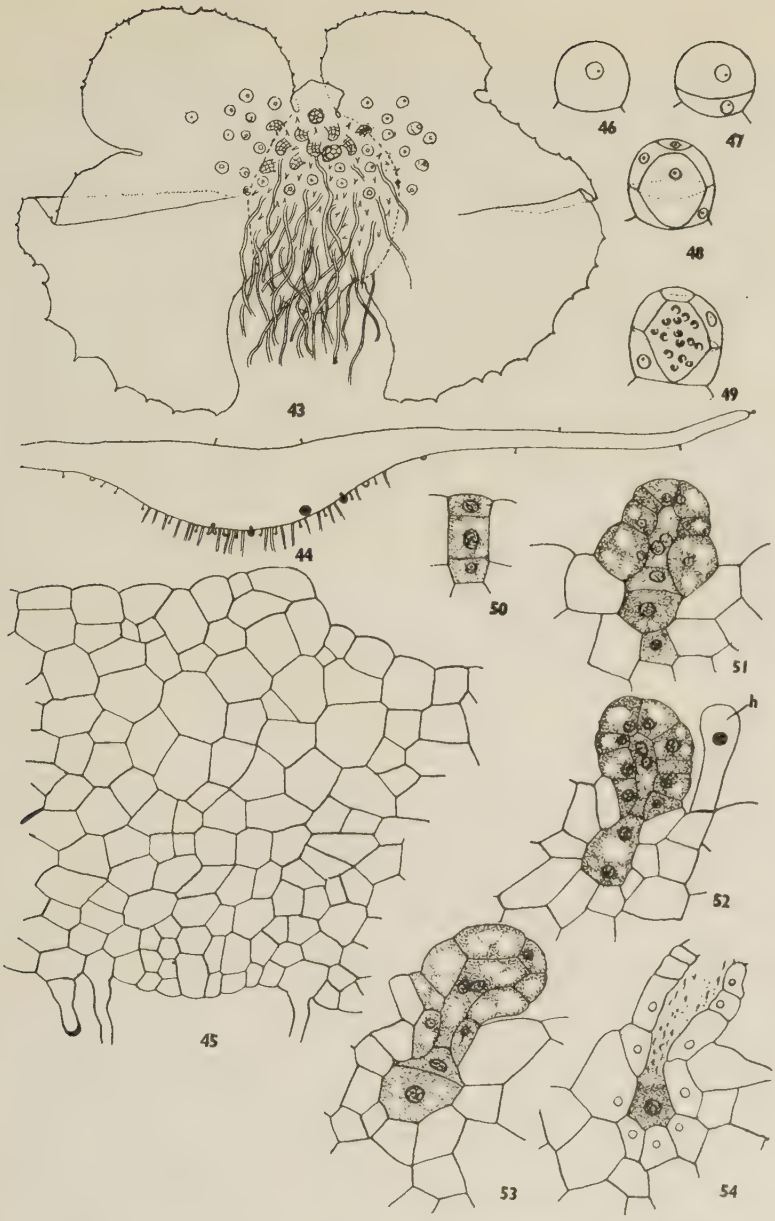
Gametophyte

The spores mature by October (at least in the tropical forests of Assam) and the gametophyte grows during the winter. Commonly, the spores germinate in the humus collected within the crevices on the barks of trees and also in the humus collected by the leaf bases of the mother plant. Under laboratory conditions they germinate within two weeks in soil cultures. The exine ruptures along the longitudinal scar and the intine protrudes out as the germ papilla which develops chloroplasts very soon. Further development of the filament and the formation of the cordate prothallus is normal (Figs. 34-39). The rhizoid may be late in development. A two-sided apical cell is established when the filament is 3 or 4 cells long (Fig. 36), but is soon replaced by a group of marginal meristematic cells which eventually become located in an apical notch (Fig. 39). In insufficient light the germ tube may grow into a very long filament, sometimes branched (Fig. 35). If unfavourable light conditions set in early in the development of the plate, the plate reverts to the filamentous stage again. As in *Drynaria* (Nayar & Kachroo, 1953) the marginal cells of the young prothallus develop unicellular papillate hairs (Fig. 38). The mature prothallus is cordate bearing sex organs and rhizoids on the lower surface of the midrib (Fig. 43). The lobes are composed of uniformly thin-walled, densely chlorophyllous, polygonal cells arranged in more or less radiating rows. The papillate hairs occur densely on the lower surface of the midrib (between sex organs and rhizoids) and on the margin of the lobes, while they are sparsely distributed on the surface of the lobes. They originate as a protrusion of one of the superficial cells which elongates and is cut off as a hair (Fig. 40, *h*). Rarely chloroplasts are met with in young hairs. When young, the hairs have a swollen tip with a prominent nucleus surrounded by a few vacuoles on all sides (Fig. 41). In older hairs the chloroplasts degenerate and the swollen apex of the hair turns brownish with an extra cellular cap-like covering and finally wither off in very old prothalli. In old prothalli the cell that bears the hair and the adjacent cells may grow outward forming a small lobe at



FIGS. 31-42 — Fig. 31 T.s. photosynthetic region of lamina (*s*, stoma). × 160. Fig. 32. Mature sporangium attached to sorus. × 160. Fig. 33 a, b. Spore. × 360. Figs. 34-39. Stages in the development of prothallus (*h*, hair initial). 34-38 × 160; 39 × 80. Fig. 40. Apical meristem of mature prothallus. × 160. Fig. 41. Marginal hair from young prothallus. × 260. Fig. 42. Same, from old prothallus. × 260.

the top of which the hair becomes located (Fig. 42). The midrib of the fully grown prothallus is about 10 to 13 cells thick (Figs. 44, 45), composed of uniformly thin-walled irregular cells. The development of the sex organs is of the



FIGS. 43-54 — Fig. 43. Ventral view of fully grown prothallus (area enclosed by dotted line indicates midrib). × 8. Fig. 44. Portion of t.s. mature prothallus showing midrib and one wing. × 16. Fig. 45. Same, portion of midrib. × 160. Figs. 46-49. Stages in antheridial development (optical sections). × 260. Figs. 50-54. Stages in archegonial development (*h*, hair). × 260.

usual type in higher ferns. The antheridia are developed earlier, at a period of about two months after the germination of the spores. Antheridial development follows the pattern described by Davie (Davie, 1951). Stages in antheridial develop-

ment are figured (Figs. 46-49). The antheridium is globose and sessile. The opercular cell remains single and is thrown off bodily to liberate the sperms.

Archegonia are formed in normally growing prothalli when they are 3 to 4 months old. Both antheridia and archegonia are seen intermingled in earlier stages. A few stages in the development of the archegonium are figured (Figs. 50-54). The neck of the archegonium is composed of four tiers of cells and is usually 4 to 5 cells in height. The neck canal cell is binucleate, with a prominent vacuole towards the slightly dilated apex in mature archegonia. Small vacuoles are regularly distributed surrounding the egg nucleus. Irregularly arranged vacuoles are met with in the neck cells. The neck is sharply curved either towards the base of the prothallus or towards one side. During embryo formation the venter region of the archegonium forms a jacket, 4 to 6 layers thick, and with dense protoplasm.

Discussion

Pseudodrynaria coronans was recognized by earlier taxonomists as a species of the complex genus "*Polypodium*" (*Polypodium coronans* Wall.). Christensen (1934, 1938) gave it the generic status and included it under the tribe Pleopeltideae, sub-family Polypodioideae, section Lepidopterids of the family Polypodiaceae. Ching (1940) recognizes the genus and places it in the tribe Phymatodeae of the sub-family Pleopeltideae, family Polypodiaceae Presl. (*sensu propria*). He considers the genus to be more evolved than *Phymatodes, Selliguea, Microsorium, Colysis, Leptochilus* and *Arthromeris*, but more primitive than *Drynaria, Pyrrosia*, and *Drymoglossum*. Copeland (1947) includes *Pseudodrynaria* along with *Drynaria, Drynariopsis* and a few others in his group Drynarioids as a branch line of evolution from *Microsorium*. Holttum (1949) includes "*Drynaria* and its allies" in his Polypodiaceae (*sensu stricto*), grouped along with *Microsorium, Colysis* and *Leptochilus*. He considers Drynarioid ferns to be derived from *Microsorium* "by a special development of the base of the frond".

The rhizome in the genus is creeping, fleshy, short and stout, evidently correlated with the humus-collecting habit. In the related genera and species which have lost this habit (*Photinopteris, Holostachyum, Aglaomorpha pilosa*, etc.), the rhizome is comparatively slender. The peltate, water-absorbing paleae covering the rhizome are an adaptation to the epiphytic habit as in *Pyrrosia* and *Pteropsis*. The margin of the paleae is dentate as in *Drynariopsis, Drynaria quercifolia, Pteropsis* and species of *Pyrrosia*. In this respect *Pseudodrynaria* is more advanced as compared to species of *Drynaria* like *D. propinqua, Holostachyum* and *Photonopteris* which have paleae with ciliated margin.

The "dictyostelic" nature of the vascular system of the rhizome is attained by development of secondary perforations in a gutter-shaped siphonostele and not by overlapping of leaf gaps. The leaves are borne only on one side and that too in a single row. Moreover, the leaf gaps are small and end far behind the next leaf trace.

Usually in forms with entire gutter-shaped stele, like the trailing species of *Adiantum*, the leaf traces are given off from either margin alternately so that there are two rows of alternating leaves. But because of the infolding of the margins in *Pseudodrynaria*, as described above, the two margins come to lie almost in the same plane, so much so that the leaf traces originating alternately will themselves be in a single row.

The vascular strands supplying the leaf are arranged in a t.s. in the form of a horseshoe, with the concavity facing the apex of the rhizome, as in *Drynaria*. In the early juvenile leaves the leaf trace is a single strand, dividing into two just before entering the leaf. Thus at the leaf base there are two laterally placed strands as in smaller leaves of *Pyrrosia*. In later formed juvenile leaves one of the branches divides again, so that three bundles enter the leaf base, two towards the adaxial and one towards the abaxial side, a condition normal in the leaves of *Pteropsis*. The number of vascular traces entering the leaf may be conceived to be

dependent on the size of the leaf. The leaf trace in all the related genera studied originate as a single strand — at least in the early stages — splitting into two or more strands, the number depending on the size of the leaf.

The earlier formed juvenile leaves are simple and petiolate. The transition to the pinnatisect condition is associated with the increase in size of the lamina and may be considered as an advanced feature when compared to related ferns with an entire lamina. The formation of humus-collecting bases is an advanced feature. The humus-collecting basal region of the leaf of *Pseudodrynaria* persists long after the upper photosynthetic region is shed. A parallelism may be seen in *Drynaria*, where the photosynthetic leaves last only for one growing season while the humus-collecting leaves are persistent.

Venation of the lamina is strictly anaxetioid with prominent main veins and hidden reticulations, as in *Drynaria*, *Drynariopsis* and *Phymatodes*. The leaf segments are articulated to the midrib resulting in abscission on drying as in *Drynaria*.

In all drynarioid ferns (including *Pyrrosia* and *Pteropsis*) the sori are borne superficially on the veins and never on free vein endings. The arrangement of the sori in lines between main veinlets together with the pinnatifid and coriaceous nature of the leaf with its prominent main veins and hidden reticulations suggest their derivation from *Phymatodes*. The fact that in *Microsorium* the leaves are only rarely pinnatifid, are thin in texture and have sori irregularly distributed over the surface of the leaf are characters that are strongly against the consideration of *Microsorium* as the ancestral type of the drynarioid ferns, as suggested by Holttum.

Polypodiaceous paraphyses are found in the sori. Paraphyses are absent in *Drynaria* (except in *D. rigidula*, separated by J. Smith into the section Poronema).

The prothallus is massive when compared to those of *Drynaria*, *Pteropsis*, *Pyrrosia* and other advanced Leptosporangiates. It is rather slow in development, usually taking 2 to 3 months or more to produce the archegonia. This slow rate of growth cannot be considered to be a primitive feature, but it may be an adaptation to the habit. A short germ tube (more or less 4 cells long) is the usual rule during germination of spores. Unicellular club-shaped hairs occur on the margin and lower surface of the midrib. In *Drynaria* the hairs are restricted to the margin but are of the same type. In the mature prothallus *Pseudodrynaria* shows very close resemblance to *Pyrrosia* and *Drynaria*.

Summary

The rhizome of *Pseudodrynaria coronans* (Wall.) C. Chr. is fleshy, stout and creeping. The vascular system is a gutter-shaped dictyostele, with the margins of the gutter folded inwards so as to become juxtaposed towards the middle of the concave side. Epidermal appendages are peltate paleae with dentate margin and stalk probably capable of absorbing water.

Leaves are in a single row on the dorsal surface of the rhizome and have a humus-collecting, expanded basal region with a wavy margin and a photosynthetic, pinnatifid upper region. Lobes of the latter region are articulated to the midrib and are abscissile. The basal region of the leaf and the whole midrib persists. Venation is typically anaxetioid. Sori are non-indusiate, usually compital, of the mixed type, with paraphyses between the sporangia, and occurring at the intersections of the veinlets all over the dorsal surface of the leaf, in rows between the main lateral veins of the segments.

Spores are bilateral, hyaline, with sparsely spinulescent exine and a median longitudinal scar. During germination a 3-4-celled filament is formed as a rule. Growth of the prothallus in the earlier stages is by means of a two-sided apical cell, but it is soon replaced by a transverse meristem. Mature prothallus is cordate, two or more times broader than long, with a massive midrib 10-13 cells thick, and with club-shaped unicellular hairs on the margin and the ventral surface of the midrib. Sex organs are borne

ventrally on the midrib. Antheridia and archegonia may be intermingled at first.

The relationship of the genus is discussed.

Thanks are due to Dr. Alma G. Stokey for her valuable suggestions and kind criticism of the manuscript, and to Dr. H. K. Baruah and Mr. P. Kachroo for encouragement.

Literature Cited

Ching, R. C. 1940. On natural classification of the family ' Polypodiaceae '. Sunyatsenia **5** : 1-72.

Christensen, C. 1934. " Index Filicum." Supplementum Tertium. Copenhagen.

— 1938. Filicineae. Verdoorn's " Manual of Pteridology ". The Hague.

Copeland, E. B. 1947. " Genera Filicum." Waltham, Mass., U.S.A.

Davie, J. H. 1951. Development of antheridium in Polypodiaceae. American J. Bot. **38** : 621-29.

Holttum, R. E. 1949. The classification of ferns. Biol. Rev. **24** : 267-96.

Kachroo, P. & Nayar, B. K. 1953. Studies in Pteridaceae. I. Observations on the gametophyte of some species of *Adiantum*. Phytomorphology **3** : 240-48.

Nayar, B. K. & Kachroo, P. 1953. Studies in Polypodiaceae. I. Morphology of *Drynaria* Bory. Phytomorphology **3** : 411-23.

MORPHOLOGICAL AND CYTOLOGICAL STUDIES ON *CITRUS GRANDIS* OSBECK

I. BANERJI

Department of Botany, Calcutta University, Calcutta, India

Introduction

Citrus grandis, commonly known as " Shaddock " and believed to be a native of Malaya, is characterized by simple leaves with winged petioles (Banerji, 1952) and hesperidia which are over six inches in diameter. Two varieties are commonly recognized, one with white and the other with red pulp. In West Bengal, flowering commences towards the beginning of December and continues up to the end of January. The fruit takes about six months to mature.

The genus *Citrus* has received considerable attention from embryologists. Braun (1860) and Strasburger (1878) recorded the occurrence of polyembryony, followed by the more detailed work of Osawa (1912). Souèges (1926) has given an account of the embryogeny of *Ruta*. The embryology of several members of the family has been studied by Mauritzon (1935), and Chakravarty (1935, 1936) has recorded polyembryony in *Murraya koenigii*, *M. exotica* and *Aegle marmelos*. Bacchi (1943) has described briefly the development of the female gametophyte in *Citrus paradisi* and *C. aurantium*, and has explained the cause of occurrence of two non-identical hybrids from the same seed.

The cytology of *Citrus* has been studied by Longley (1925), Frost (1925, 1938) and others. The chromosome numbers of all the cultivated species and most of their varieties have been determined. In a few cases tetraploid forms have also been recorded.

Materials and Methods

The material for the present investigation was obtained from a plant growing

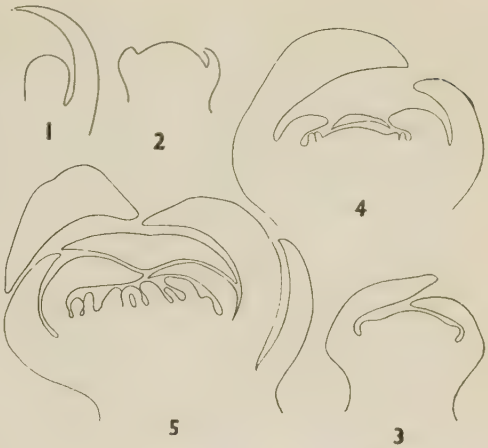
in the University College compound. Material fixed between 2 and 3 p.m. showed division stages of the pollen mother cells. Nawaschin's and Levitsky's fluids were used for fixation, and the material was dehydrated, cleared and embedded in the usual way. Sections were cut at a thickness of 8 to 16 microns. Newton's Gentian violet iodine was used for cytological studies, and for embryological work Heidenhain's iron-alum haematoxylin, with or without Orange G, was preferred.

Observations

Development of the Flower — The inflorescence of *C. grandis* is an axillary panicle. In the earliest stages the flower appears like a dome-shaped structure in the axil of a bract (Fig. 1). This soon increases in size and from its periphery arise the five sepals (Fig. 2). The petals differentiate next (Fig. 3). In older stages some of the epidermal cells, specially those occurring towards the tip, grow out as short papillate processes with ovate ends. The stamens appear as papillate processes (Fig. 4) which soon differentiate into anthers and filaments. The latter soon become united into a number of separate strands resulting in a polyadelphous condition. Subsequently a conspicuous raised disc becomes differentiated above the thalamus on the top of which several carpel primordia develop (Fig. 5). They remain separate in the initial stages, but soon their margins project inwards to form the multilocular ovary, while the tips unite to form the solid style and capitate stigma. The style is characterized by the presence of conducting canals whose number corresponds to the ovarian chambers. The stylar canals are lined by elongated cells and are continuous from the tip of the stigma to the ovarian chamber.

Lysigenous oil glands occur in the sepals, petals, outer region of the ovary and the stigma.

Microsporogenesis and Male Gametophyte — The archesporial cells are hypodermal in origin. They divide periclinally to produce an outer layer of parietal cells and an inner of sporogenous



Figs. 1-5 — Stages in the development of the flower (explanation in text). × 15.

cells. By further divisions of the primary parietal cells, four layers are produced of which the innermost forms a secretory tapetum (Fig. 6). The outermost layer functions as the endothecium which later shows the characteristic fibrous thickenings. In the mature anther, the middle layers become greatly flattened and crushed.

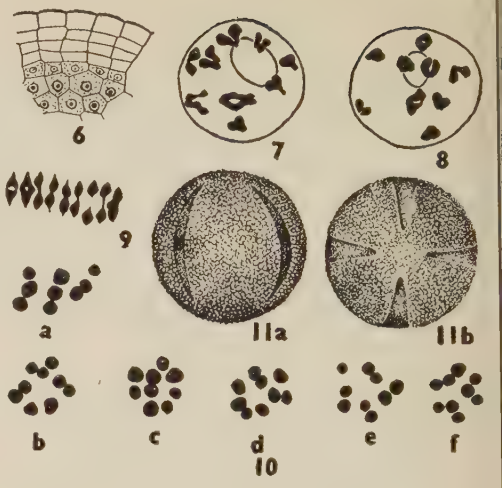
The tapetal cells are uninucleate in the early stages of development of the pollen mother cells. They divide during synizesis and most of them become binucleate. In some cells, however, cytokinesis follows karyokinesis, resulting at places in two or three layers of tapetal cells which also become binucleate.

The primary sporogenous cells divide repeatedly to form a large number of microspore mother cells. Meiosis presents no unusual features. At diakinesis nine bivalents are seen, of which three are attached to the nucleolus (Fig. 7). In the smaller bivalents the chromosomes lie side by side or are sometimes held by a terminal chiasma, while in the larger bivalents the chiasmata are often at the ends so that ring-like bivalents are produced (Figs. 7, 8). Towards the end of diakinesis the nucleolus shows a progressive reduction in size and finally disappears. The bivalents undergo further condensation in size so that they appear as spherical bodies. They move towards

the centre of the nucleus forming groups or associations. This is the beginning of a secondary association of chromosomes which is also observed in metaphase II. Fig. 10 *a-f* illustrates some of the different associations that have been observed.

An analysis of the different types of secondary associations as observed in metaphase I is presented below.

No. of cases	Number of bivalents in association			Types of associations
	1	2	3	
3	7	1	—	1 (2) + 7(1)
5	3	3	—	3 (2) + 3 (1)
5	1	4	—	4 (2) + 1 (1)
8	1	1	2	2(3) + 1(2) + 1(1)
2	3	3	—	3 (2)+3 (1)
5	—	—	3	3 (3)
2	—	3	1	1 (3)+3 (2)
5	5	2	—	2 (2)+5 (1)
4	6	—	1	1 (3)+6 (1)
5	4	1	1	1 (3)+1 (2)+4 (1)
6	2	2	1	1 (3)+2 (2)+2 (1)
50				



Figs. 6-11 — Fig. 6. Differentiation of the sporogenous cells in the anther. × 125. Figs. 7, 8. Early and late diakinesis. × 1600. Fig. 9. Disposition of bivalents in metaphase I. × 1600. Fig. 10, *a-f*. Different types of secondary associations at prometaphase. × 1600. Fig. 11, *a*, *b*. Pollen grains. × 600.

The maximum number of association is 3(3), i.e. three groups of 3, making three separate associations. The basic number, therefore, appears to be 3.

Side views of metaphase show that the bivalent chromosomes group themselves regularly on the equatorial region of the spindle (Fig. 9). The movement of the univalents is regular and laggards have not been noted. On reaching the poles the chromosomes organize into interkinetic nuclei. They appear to have become somewhat elongated and show the split for the second division. The chromosomes retain their individuality at this stage. The second division is normal. Daughter nuclei are soon organized, their arrangement being mostly tetrahedral. Cytokinesis takes place by a process of furrowing.

The pollen grains are tetracolpate, a germ pore being situated in the centre of each of the furrows (Fig. 11, *a*, *b*). When dry, they are somewhat ellipsoidal but assume a spherical form when mounted in lactic acid and show an average diameter of 34 microns. The exine is granulated (Fig. 11, *a*, *b*). The mature pollen grains are trinucleate, the vegetative nucleus being larger than the male nuclei.

Ovule — The ovary is multicarpellary and situated on a conspicuous disc which is slightly raised at the edges. Linear rows of ovules occur in each loculus on the axile placenta. Along with the differentiation of the ovules, the outer cells of the placental tissue grow out in the form of unicellular hairs (Fig. 27) which persist up to the time of fertilization.

The ovules are bitegmic. They arise as papillate processes from the placental tissue which soon curve upwards to take up an anatropous form. The inner integument arises first followed very soon by the outer. By rapid growth the latter soon encloses the former. The inner integument is composed of four layers of cells except in the micropylar region where it is about six cells thick. The outer integument is massive and is six to eight layered. Both the integuments take part in the formation of the micropyle.

The ovules are crassinucellate. The nucellar epidermis divides periclinally to

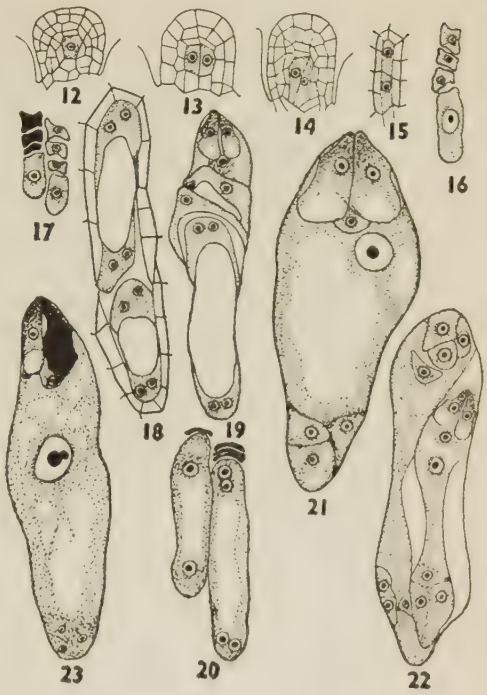
form an epidermal cap (Figs. 12-14), which becomes multi-layered during the later stages of embryo sac development. The cells at the chalazal region of the ovule form a hypostase, below which terminates the vascular strand of the ovule.

Megasporogenesis and Embryo Sac— The earliest stages in the differentiation of the archesporial cell were not observed. The megaspore mother cell, when first detected, was found to be located in the fourth layer of the nucellus, but very soon it becomes more deeply placed inside the nucellus on account of the division of the epidermal and the subepidermal cells. The presence of two or more megaspore mother cells has been observed in a number of preparations. These may lie either side by side or one below the other (Figs. 13, 14). A linear tetrad of megaspores is produced of which the chalazal alone functions (Fig. 16). The degeneration of the non-functional megaspores takes place from above downwards. Due to the activity of more than one megaspore mother cell, very often two or more linear tetrads are produced (Fig. 17).

The nucleus of the functional megaspore undergoes three successive divisions to produce an eight-nucleate embryo sac. Many preparations showed the presence of two uninucleate, binucleate, quadrinucleate or mature embryo sacs, lying either side by side or superimposed (Figs. 18, 20, 22). In rare instances, three or four embryo sacs in different stages of development were seen inside the same nucellus (Fig. 19). The mature embryo sac shows the normal organization. The synergids are slightly hooked with basal vacuoles. The egg is suspended below the synergids and occupies a central position. The secondary nucleus lies close to the egg and the antipodals are ephemeral (Fig. 21).

Pollination and Fertilization — Anthesis takes place at about eight in the morning and the receptivity of the stigma is indicated immediately afterwards by the presence of a viscid secretion on its surface. Pollination normally occurs soon, mostly by the help of insects. Scrapings taken from the stigmas, thirty minutes after pollination, showed that the pollen grains germinate immediately. This has also been verified *in vitro* by germinating pollen grains in sugar-agar medium. They lose their viability very soon. Twenty-four hours after anthesis, the viability was reduced to 10 per cent, while after 48 hours, it was nil.

The passage of the pollen tube through the style is mostly intercellular and not through the stylar canals. It is only in the stigmatic region that some pollen



Figs. 12-22 — Fig. 12. The differentiation of the megaspore mother cell in the fourth layer of the nucellus; note division of the epidermal cells. × 110. Figs. 13, 14. Two megaspore mother cells lying side by side and one above the other. × 110. Fig. 15. Dyad. × 110. Fig. 16. Linear tetrad of megaspores. × 175. Fig. 17. Two linear tetrads of megaspores lying side by side. × 175. Fig. 18. Two embryo sacs at 4-nucleate stage lying one above the other. ×175. Fig. 19. Superimposed multiple embryo sacs in different stages of development. × 175. Fig. 20. Two embryo sacs lying side by side, one at the 2-nucleate and the other at the 4-nucleate stage. × 175. Fig. 21. Mature embryo sac. × 225. Fig. 22. Two mature embryo sacs lying side by side. × 225. Fig. 23. Double fertilization. × 225.

tubes have been seen to enter the stylar canals. The pollen tube enters the embryo sac through the micropyle and disorganizes one of the synergids. Syngamy and double fertilization appear to take place simultaneously (Fig. 23).

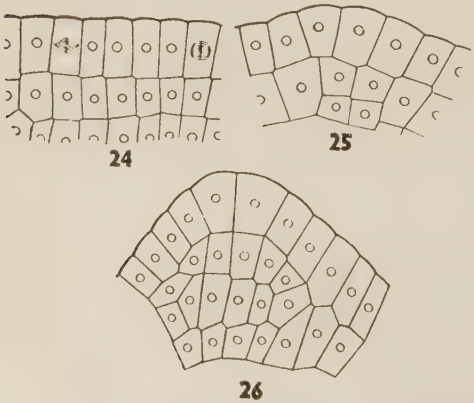
Endosperm and Embryo — The endosperm is of the nuclear type, although later it becomes cellular.

The early stages in the development of the embryo have not been studied. With the differentiation of the cotyledonary lobes the dermatogen, periblem and plerome become clearly defined. The mature embryo consists of two large cotyledons which completely fill up the seed and an axis bearing the plumule and the radicle. The plumule is somewhat dome-shaped. The radicle shows the presence of a root cap composed of a varying number of cell layers. The short suspensor is four to six cells thick.

Juice Sacs — The juice sacs extend into the loculus. Their development is similar to that in the Eureka lemon studied previously by Ford (1942).

The juicy emergences originate after fertilization from groups of 6 to 10 densely cytoplasmic cells lining the inner layer of the pericarp (Fig. 27). The epidermal cells first increase in length and then divide by anticlinal walls (Fig. 24). The sub-epidermal cells also increase in length and divide at first periclinally (Fig. 25), and then in all planes. This results in the formation of papillate processes which protrude inside the loculi (Figs. 26, 27). They rapidly increase in length and gradually fill up the chamber completely (Fig. 28). They are somewhat swollen near the distal ends, and have pointed tips and elongated stalks (Fig. 28). Transverse and longisections of the developing emergences during various stages of growth show that they are delimited externally by a layer of epidermal cells which continuously divide in an anticlinal plane. In the central region of the swollen portion of the emergence, there occur comparatively larger cells with minute intercellular spaces and thin hyaline walls. Their protoplasts show the presence of large vacuoles. These are the actual gland cells which on maturity become disrupted in the central region and form lysigenous cavities (Fig. 28).

On separating the individual carpels of the mature fruit, they are seen to be covered by a thin membranous layer, which is part of the wall of the confluent carpels and is characterized by a longitudinal disposition of the cells.



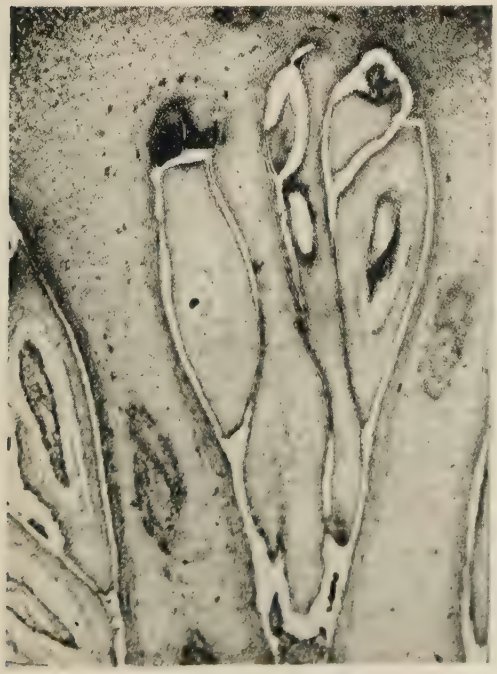
Figs. 24-26 — Fig. 24. Division of epidermal cells lining the pericarp. × 300. Fig. 25. Initial stages in the development of the juicy emergences; note periclinal divisions of the hypodermal cells. × 300. Fig. 26. The development of the papillate processes is due to the localized activity of a group of hypodermal and sub-hypodermal cells. × 300.

Discussion

The chromosome numbers of almost all the varieties of cultivated oranges have been determined. Strasburger (1907) and Osawa (1912) have recorded the presence of 8 pairs of chromosomes at diakinesis. Osawa (1912) further stated that the somatic number is " more than 14, probably 16 ". Frost (1925), Krug (1943) and others have definitely established that the haploid and diploid numbers are 9 and 18 respectively, which has been supported by the results of this investigation. Krug and Bacchi (1943) also found triploid forms.

The cytology of the genus *Citrus* has been previously studied by Longley (1925) who found irregularity in chromosome pairing and distribution at meiosis. No evidence of such irregular behaviour of chromosomes was obtained in the course of the present study. The secondary asso-

27



28

ciation of the chromosomes, a marked feature of meiosis, has not been recorded by previous investigators. The basic number, as determined from the maximum association, is 3, and *C. grandis*, therefore, appears to be a secondary polyploid.

The presence of more than one megaspore mother cell in the ovule has been previously recorded in a number of genera of the family. Bacchi (1943) figures only one megaspore mother cell in *C. paradisi* and *C. aurantium*, but notes the presence of supernumerary embryo sacs in different stages of development. The presence of multiple embryo sacs has been noted in *Poa*, *Hiptage*, *Medicago* and many other plants. Their origin might be due to (1) the functional activity of megaspores of different tetrads, (2) the activity of different megaspores of the same tetrad, or (3) from nucellar cells as observed in *Poa pratensis*. The present investigation shows that the origin of multiple embryo sacs in *C. grandis* may be due to the activity of the megaspores of the same or different linear tetrads.

In all the plants of Rutaceae so far studied, the development of the embryo sac has been found to be of the "Polygonum" type, excepting *Xanthoxylum*, in which, according to Mauritzon (1935), the development might correspond to *Polygonum*, *Allium* or *Adoxa* type. Mauritzon's observations require corroboration.

Polyembryony in *Citrus* was observed as early as 1878 by Strasburger. Since then, it has been recorded in a large number of plants of the family Rutaceae, particularly in *Xanthoxylum* sp., *Murraya koenigii*, *Murraya exotica* and *Aegle marmelos*. These are instances of nucellar polyembryony. Webber and Batchelor (1946) and Leroy (1947) have also recorded the occurrence of adventive embryony in *Citrus*. Bacchi (1943) observes that the occurrence of two or more gametophytes in the same ovule might explain the origin of two zygotic embryos sometimes seen in a seed.

←—

FIGS. 27, 28 — Fig. 27. T.s. ovary showing a single loculus and an ovule inside: *h*, placental hairs; *j.e.*, initial stages in the development of the juicy emergences. × 100. Fig. 28. Juicy emergences inside the ovarian cavity. × 20.

Polyembryony has not been detected in the present study. It should be mentioned that the material used in this investigation has been obtained wholly from a single plant growing in the college compound, which might be a monoembryonic variety.

Summary

1. The floral organs of *Citrus grandis* develop in the following sequence: sepals, petals, stamens and carpels. The ovary is multicarpellary and the style shows the presence of stylar canals.

2. The anther wall is made up of the epidermis, endothecium, two middle layers and the tapetum. The tapetal cells become binucleate.

3. Meiosis is normal. The haploid number of chromosomes is 9. Secondary association of chromosomes has been observed and the maximum association has been found to be 3. Pollen formation is simultaneous. The pollen grains are trinucleate and tetracolpate and have a granulated exine.

4. The ovules are bitegmic and crassinucellate.

5. The megaspore mother cell was first noted in the fourth layer of the nucellus. A tetrad of megaspores is formed and the chalazal cell develops into an eight-nucleate embryo sac. Two or more megaspore mother cells, linear tetrads, two-, four- or eight-nucleate embryo sacs, lying close together inside the same nucellus, are of frequent occurrence. The mature gametophyte is of the 'Polygonum' type. The antipodals are ephemeral.

6. The endosperm is of the nuclear type but later becomes cellular. The embryo has a short suspensor and the cotyledons are slightly unequal. The plumule is dome-shaped and the radicle shows the presence of a root cap.

7. The juicy emergences of the fruit develop from the epidermal and subepidermal cells of the inner layer of the pericarp. They form multicellular structures, which grow rapidly and have pointed ends. Lysigenous cavities develop in their central region.

Thanks are due to Professor P. Maheshwari for helpful criticism.

Literature Cited

Banerji, I. 1952. The unifoliate compound leaf of *Citrus grandis*. Bull. Bot. Soc. Bengal **6**: 1-5.

Bacchi, O. 1943. Cytological observations in *Citrus*. III. Megasporogenesis, fertilization and polyembryony. Bot. Gaz. **105**: 221-225.

*Braun, A. 1860. Über Polyembryonie und Keimung von *Caelebogyne*. Abh. Ak. Berlin, phys. Kl. 107-263.

Chakravarty, R. S. 1935. Polyembryony in *Murraya koenigii*. Curr. Sci. **3**: 361-362.

— 1936. Nucellar polyembryony in Rutaceae. Curr. Sci. **5**: 202-203.

Ford, E. S. 1942. Anatomy and histology of Eureka lemon. Bot. Gaz. **104**: 228-304.

Frost, H. B. 1925. The chromosomes of *Citrus*. Genetics **15**: 1-3.

— 1938. The genetics and cytology of *Citrus*. Curr. Sci., Special number: 24-27.

Krug, C. A. 1943. Chromosome numbers in the subfamily Aurantioideae with special reference to the genus *Citrus*. Bot. Gaz. **104**: 602-611.

— & Bacchi, O. 1943. Triploid varieties of *Citrus*. J. Heredity **34**: 276-283.

*Leroy, J. F. 1947. La polyembryonie chez les *Citrus*. Son interet dans la culture et amélioration. Rev. Int. Bot. Appl. et d'Agr. trop. **27**: 483-495.

Longley, A. E. 1925. Polycarpy, polyspory and polyploidy in *Citrus* and *Citrus* relatives. Genetics **15**: 347-351.

Mauritzon, J. 1935. Über die Embryologie der Familie der Rutaceae. Svensk bot. Tidskr. **29**: 319-349.

Osawa, I. 1912. Cytological and experimental studies in *Citrus*. J. Coll. Agri. Imp. Univ. Tokyo **4**: 83-116.

*Strasburger, E. 1878. Über Polyembryonie. Jenaische Z. **12**: 647-670.

*—1907. Über die Individualität der Chromosomen und die Pfropfhybriden-Frage. Jb. wiss. Bot. **44**: 482-555.

*Souèges, R. 1926. Developpement de l'embryon chez le *Ruta graveolens*. Bull. Soc. Bot. France **73**: 245-260.

Webber, H. J. & Batchelor, L. D. 1946. "The Citrus Industry. I: History, Botany and Breeding." Univ. of California Press.

*Not seen in original.

SEEDLING DEVELOPMENT AND HAUSTORIAL SYSTEM OF *LORANTHUS MICRANTHUS* HOOK. F.

BARBARA P. MENZIES
Bedford College, University of London, England

Introduction

The haustorial systems of several members of the Loranthaceae have been described in some detail. Considerable diversity of structure has been found and attempts have been made to categorize haustorial types (Engler & Krause, 1935). The present paper describes the hitherto uninvestigated development and structure of the haustorial system of *Loranthus micranthus* Hook. f., the single endemic species of the genus found in New Zealand. It will be shown that, in haustorial structure, it closely resembles a group of species of the genus *Struthanthus*.

The fruits of *Loranthus micranthus* ripen in the autumn and are about $\frac{1}{3}$ in. long and bright yellow. The fruit is a pseudocarp, the fleshy part of which breaks down into a viscin which serves to cement the single enclosed seed to the host. *L. micranthus* seems to be entirely dependent on bird agency for dispersal. The fruit, when ripe, is surrounded by a non-sticky skin. Observations show that when a bird bites or sucks at a fruit, the seed, surrounded by the viscin, may be extruded suddenly from the fruit skin, and either fall on to the vegetation beneath or stick to the bird's beak and be wiped off later on to a new host. Alternatively, the seed may be eaten whole and excreted with the faeces: germinating seeds have been found stuck to the branches of trees in the excrement of birds. In all these cases, the seeds begin to germinate at once.

About a month after dissemination, the radicle appears, bends towards and enters the host branch. The first two leaves develop within the next two or three months and the second pair by the end of the first year (Plate I, Fig. 3). The main axis, short at first, eventually grows out and branches. Further shoots arise adventitiously from the base of the main stem forming the adult bushy plant. The cotyledons remain within the seed. As the parasite grows older, the portion of the host stem attacked by it usually becomes much swollen.

Some plants produce aerial roots. These arise from the base of the seedling close to the junction with the host (Plate I, Fig. 3) and grow along the surface of the host branch, sending in haustoria at intervals (Plate I, Fig. 1). Leafy shoots may arise from these aerial roots, usually opposite haustoria. Aerial roots branch, but only very infrequently, and possibly only when the original growing point is injured. Sometimes the roots lose contact with the host and twine about in the air. By a process of self-parasitism, when haustoria from one root grow into another, a very complicated " root system " is developed (Plate I, Fig. 2).

L. micranthus has a wide host range, occurring on both angiosperms and gymnosperms, native and introduced (Laing & Blackwell, 1940). In the Auckland Province, the writer has observed the following trees bearing adult plants of the mistletoe: *Podocarpus totara*[1], *Pittosporum tenuifolium, Melicytus ramiflorus, Schefflera digitata, Aristotelia racemosa, Fuchsia excorticata, Rubus australis* and *Corokia cotoneaster*. *Loranthus* seeds, however, germinate readily on any suitable surface on which they happen to fall and are able to live for some months on food reserves. Consequently, any shrub, herb or even fern growing under an adult parasite often bears numerous young *Loranthus* seedlings. Sections show that the haustoria of such

1. The nomenclature throughout is that of Cheeseman, 1925.

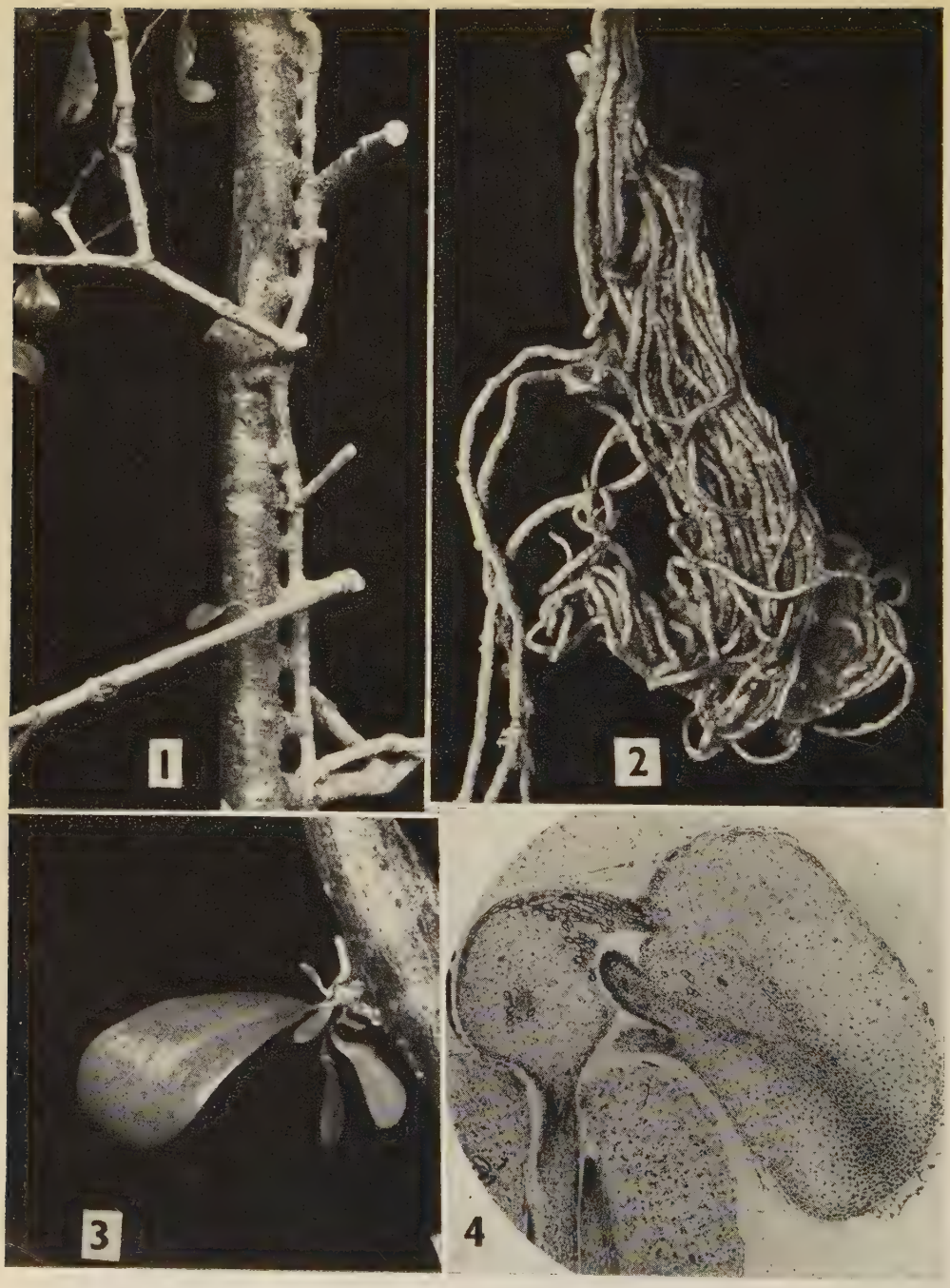


PLATE I

FIGS. 1-4 — Fig. 1. Aerial root growing along the under surface of host branch and sending in haustoria at intervals. × ½. Fig. 2. A tangled mass of aerial roots, many of which are joined together with haustoria. × ½. Fig. 3. Two-year old seedling with an aerial root growing out along the host branch. Fig. 4. L.s. axis of seedling before development of holdfast and haustorium. Endosperm of seed and embedded cotyledons are at the left. At this stage the "radicle" of the seedling has a subterminal meristem. × 37.

seedlings often penetrated the host tissue and, in some cases, e.g. *Hebe salicifolia*, actually reached the xylem. Further development is prevented in some way, for adult plants never occur on such hosts. Seedlings have been found on the leaves of both congenial and uncongenial host; sections of these showed that the haustoria had penetrated the leaf, distorting and sometimes destroying the tissues. No xylem connections were found on leaves.

Anatomical Investigations

For most of the anatomical work material was preserved in formalin-alcohol (4 per cent formalin in 70 per cent alcohol). Much of the woody material, especially older parasites growing on woody host plants, was too tough for paraffin embedding and this was sectioned in a Spencer sliding microtome. Steam was used to soften some of the material but did not give good results with delicate, easily distorted haustoria. Such material was successfully treated by soaking it in pure glycerine for a month before sectioning.

For staining, a combination of safranin (2 per cent in 3 per cent aniline oil in 95 per cent alcohol) and fast green (½ per cent in absolute alcohol) gave the best results.

Development of the Seedling — This section deals only with those parts of the parasite found *outside* the host.

Seeds can be germinated away from the host on damp cotton wool. The hypocotyl, which is bright green, grows out to a length of about 3-4 mm. It is always strongly curved, making sectioning difficult. The tip then swells to form the holdfast and at its apex appears a small, yellowish area, in the centre of which is a somewhat depressed, brownish spot, the attachment region. From this grows out a tiny, pale brown, irregular, lobed structure — the primary haustorium (Fig. 1). No further growth takes place unless contact is made with a host plant.

Sections of young seedlings before the development of the holdfast show, in the hypocotyl, four strands of meristematic tissue, which are the traces of the cotyledons and the first two leaves. Nearer the tip of the radicle, the meristematic tissue forms one central strand. This must be the radicle in the strict sense. In longitudinal sections of the radicle, the apical meristem appears to be situated at a short distance behind the tip (Plate I, Fig. 4). This resembles the structure of a typical root tip. Soon the tip of the radicle swells to form the holdfast and all resemblance to a root tip is lost.

Sections through the developing holdfast show that the superficial cells of what will be the attachment region become filled with a red staining material and later break down. This collapse of cells, coupled with continued growth of the peripheral parts of the holdfast, causes the attachment region to be depressed. The mucilaginous remains of the dead cells of the attachment region help to keep the holdfast attached to the host after the viscin surrounding the seed has weathered away.

The primary haustorium grows out from the attachment disc. The cells on the advancing edge have dense contents and many are papillate or even hair-like. There is a sub-terminal meristem (Fig. 2) which gives rise to a central strand of provascular tissue surrounded by a cortical zone of parenchyma. Meanwhile the rim of the holdfast develops to form a collar round the neck of the haustorium. Characteristic zones of collapsed cells appear in the holdfast and also between the holdfast and the haustorium. The haustorium then proceeds to penetrate the host. Further flaps of tissue like the collar develop from the neck of the haustorium and as the haustorium pushes into the cortex, portions of the epidermis and cortex of the host may be pushed up between the flaps.

The peculiar zones of collapsed cells in the holdfast are difficult to interpret. The collapse of those between the holdfast and the haustorium is presumably due to internal strains set up as the haustorium grows out. The zones in the cortex of the holdfast (Fig. 2) are very similar to those described by Thoday (1951) in the same region of the holdfast of *Viscum album*. He suggests that they may correspond to the zones of collapsed cells in the contractile roots of *Oxalis* spp. and have the same function of keeping the holdfast closely pressed against the host and

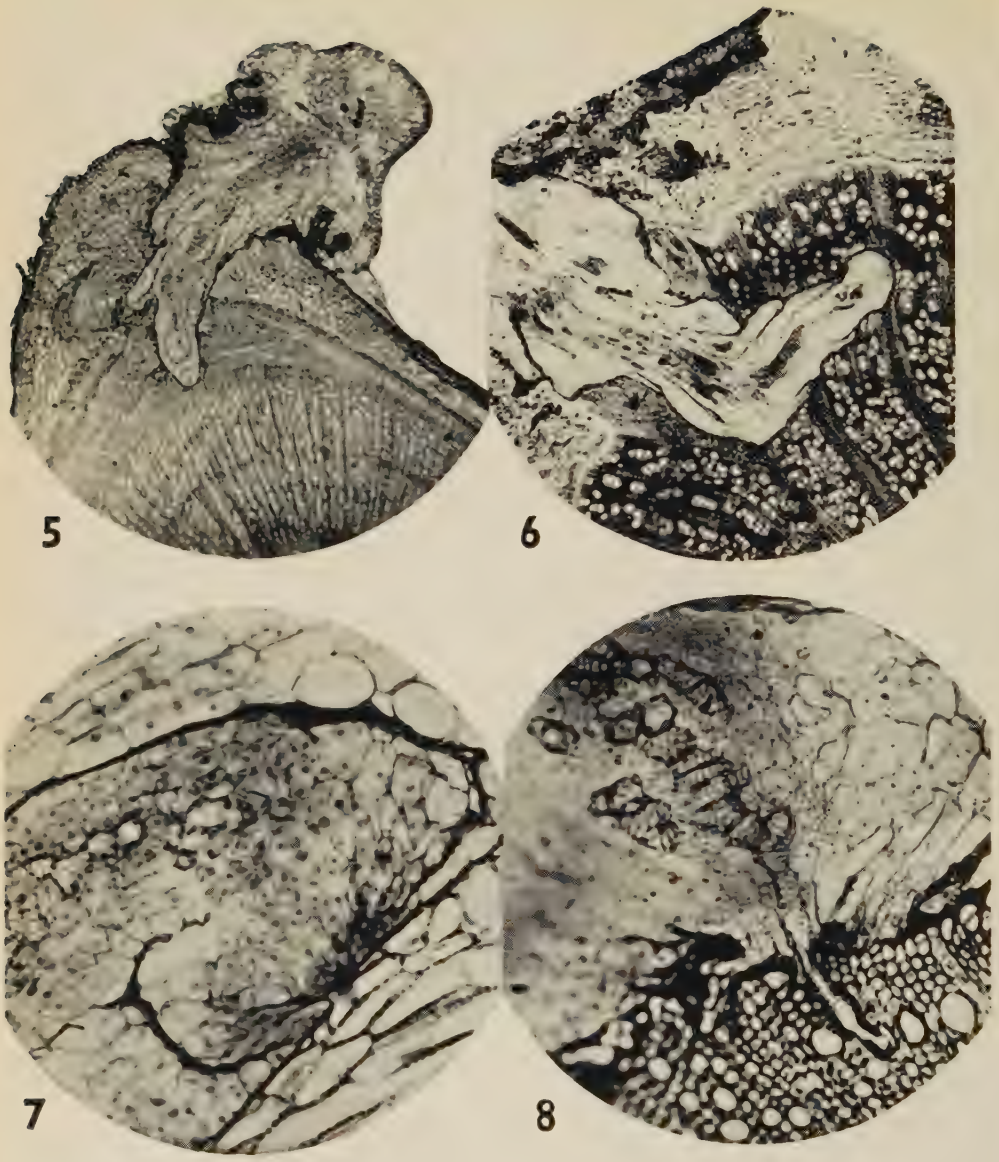


PLATE II

FIGS. 5-8 — Fig. 5. T.s. host (*Hebe salicifolia*) and parasite. The primary haustorium has grown in as far as the outer layers of the xylem. × 32. Fig. 6. T.s. host (*Schefflera digitata*) and parasite. The primary haustorium has produced a branch which travels at right angles within the hot xylem. Several zones of collapsed cells can be seen in the haustorium. × 22.s Fig. 7. T.s. young cortical meristem in an haustorium at left. An elongated cell has grown out of the haustorium into a tracheid of the host xylem (lower right). A very distinct zone of collapsed cells surrounds the cortical meristem. Host: *Pittosporum tenuifolium*. × 210. Fig. 8. A water-conducting cell from the haustorium (at left) has grown a considerable distance into the host xylem and applied itself to a vessel. The cell has then been converted into a vessel which is in direct communication with the vascular tissue of the haustorium. Host *Pittosporum tenuifolium*. × 210.

also of reinforcing the thrust of the haustorium.

Meanwhile the first two leaves have appeared and differentiation of the vascular tissues of the axis proceeds. In the hypocotyl there are four collateral bundles. In the short radicle region, the primary xylem cylinder appears to be tetrarch. The transition between the two regions is difficult to follow, but it appears that as the xylem of the hypocotyl bundles moves in towards the middle of the axis, the phloem of each of the bundles divides into two and each half moves laterally to join the half from the adjoining bundle. The change in orientation of the metaxylem with respect to the protoxylem is obscure. No endodermis could be distinguished, nor is it found in aerial roots. The radicle region is very short: lower down, in the primary haustorium, the vascular tissues become re-oriented again, usually into two bundles which appear to be collateral, with endarch protoxylem. Fig. 3 is a diagrammatic representation of the xylem of the axis of a seedling.

Secondary thickening begins early in all aerial parts of the parasite, usually by the time the second pair of leaves have developed. In the epicotyl and hypocotyl, the cambium behaves in the normal manner of stems. In the radicle region it behaves as in roots, producing a cylinder of xylem with conspicuous parenchyma rays opposite the protoxylem groups. In the primary haustorium secondary growth is confined to the two collateral bundles.

The structure of the basal parts of the axis may be further complicated by the production of aerial roots and adventitious shoots. Aerial roots are produced from the radicle region. They are mainly endogenous, arising from the inner layers of the cortex which are probably equivalent to a pericycle. Mature aerial roots resemble typical dicotyledonous roots in general structure but with a few important differences. A common apical meristem produces a massive root cap, but since the roots of *Loranthus* never penetrate soil, the root cap cells are not rubbed away but can still be seen with the naked eye as wisps of tissue adhering to the surface of the root, especially on the underside, for some distance from the tip. Root hairs are never developed. The parts of the root exposed to the light become reddish-brown in colour due to a deposit of colouring matter in the exodermis. An endodermis could not be distinguished. There are usually about ten alternating xylem and phloem groups surrounding a fairly wide pith which, in older roots, becomes strongly lignified. Secondary thickening is initiated quite early, often within 2 cm. of the tip, but the rate of radial growth is exceedingly slow. Periderm formation begins at approximately the same time. The first phellogen divisions occur on the shaded side of the root. On the exposed, coloured side, groups of densely staining cells become much elongated at right angles to the root surface to about three times their normal length. Beneath these groups of cells, the outer layer of the cortex begins to divide after the manner of a phellogen, producing conspicuous lenticels. Meristematic activity spreads round the rest of the root to form a complete phellogen. This phellogen remains active for the life of the root and produces several layers of tannin-filled phellem.

Development of the Haustorium — On entry into the host plant, the primary haustorium, a stumpy, pointed structure, travels straight through the epidermis and/or periderm, cortex, phloem and cambium until it reaches the outer layers of the xylem (Plate II, Fig. 5). Penetration seems to be effected partly by pressure and partly by enzyme action. There is a generalized compression of tissues in the path of the haustorium (Plate II, Fig. 6), but those cells in actual contact with the advancing edge are not crushed but cleanly dissolved away (Fig. 4).

The passage of the primary haustorium produces abnormalities in the host. The most noticeable effect is the stimulation of the cambium for some distance from the parasite to produce more secondary tissue, thus accounting for the hypertrophy seen in older plants. In the host stem in Plate II, Fig. 5, for example, the normal cambium had only just begun to give rise to spring wood; near the haustorium there was a zone of spring wood over 300 μ wide. This zone diminished in width and

finally disappeared at about 1·3 mm. from the haustorium. The cambium close to the parasite behaves peculiarly, producing elements oriented in all directions. The gall which eventually develops as a result of entry of a parasite may be four or five times the diameter of the unaffected stem (Fig. 5). Much of this " extra " xylem is made up of lignified parenchyma, a general feature of gall tissue (Kuster, 1930). Hypertrophy was also noted when haustoria invaded leaves.

Penetration of the host xylem occurs in late spring and early summer, six to nine months after the seeds are shed. The haustorium never advances beyond the summer wood of the previous year. It then produces a branch haustorium at right angles which travels in the latest formed xylem, more or less parallel to the cambium (Plate II, Fig. 6). The actual water-absorbing elements develop on these branches.

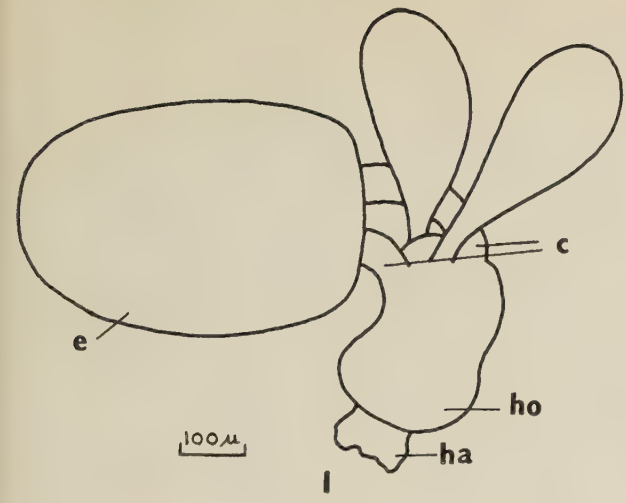
Growth of an haustorium appears to be a discontinuous process made up of a number of bursts. In each case growth is initiated by a meristem arising more or less endogenously in the old haustorium, rather after the fashion of a branch root. The occurrence of each burst can easily be recognized since it results in a zone of collapsed cells delimiting the branch from the cortex of the parent haustorium (Plate II, Fig. 6). The number of bursts can then be found by counting the number of lines of collapsed cells in the haustorium. There does not appear to be any seasonal rhythm in these bursts. In a parasite, 18 months old, there may be 6 or 8 bursts. In fact, there is nothing to suggest that growth of the hemi-parasite is not continuous throughout the year. The absence of annual rings in old stems would support this.

In a well-developed parasite the haustorial system becomes very complex. There may be three or four haustorial branches coming from either side of the primary haustorial axis (Fig. 6). Fig. 7 shows a reconstruction of a typical haustorial system. The haustoria are so delicate that they could not be dissected out of the host xylem; hence the reconstruction had to be made from serial sections. It shows the haustoria to be irregular flanges encircling the host xylem. Their irregular outline results from the sporadic type of growth. There is apparently no distinct apical growing point laying down tissues in an orderly fashion. Instead, a portion of the haustorium grows actively for a time and then ceases, growth being resumed at another point. Thus, although the haustorium appears to travel in one general direction, this movement is actually made up of a series of unconnected bursts of growth, which may extend the haustorium in almost any direction up, down or round the xylem cylinder.

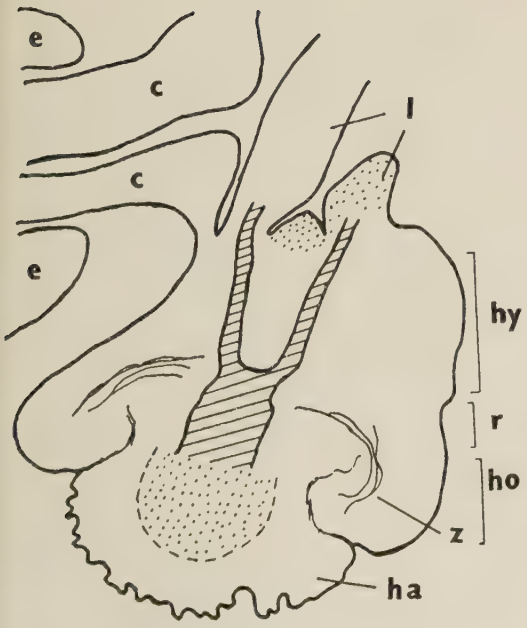
In a parasite with a succession of haustorial branches developed from the primary axis, the younger ones in the outer layers of the host xylem appear to be growing most actively, as judged by the amount of meristematic tissue present. It is fairly well established that the most recently formed xylem is the most important in conduction of water. Hence, if a water parasite is to maintain itself successfully for some time, it must continually tap these latest formed elements. In this respect, *L. micranthus* seems well adapted to its mode of life. The haustoria, in addition to being produced in the latest formed wood, also tend to move gradually outwards as they encircle the stem, evidently keeping pace with its growth in diameter. Thus contact between actively growing haustoria and new wood is maintained.

By observing the number of annual rings passed through by an haustorium as it grows outwards, it should be possible to estimate its age and also its growth rate. The host *Melicytus* is well suited to such a study because its rings are well marked and wide apart even in gall tissue. One such haustorial branch grew 1·3 cm. in one year, i.e. the time between the production of spring wood in two successive years. Another grew 2·5 cm. in two years.

The length of haustoria is probably governed by the hardness of the wood of the host. For example, the wood of *Pittosporum* is much harder than that of *Melicytus*, as judged by the ease of cutting and the structure of the xylem. Haustoria in *Pittosporum* of the same age as those in *Melicytus* (i.e. passing through the same number of annual rings) were



3

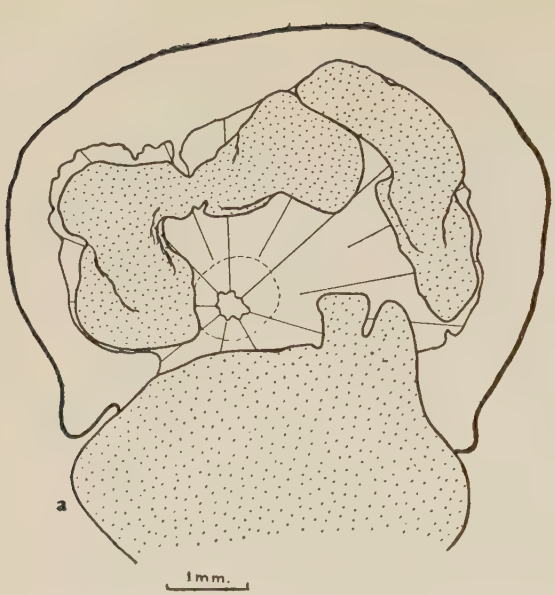


2

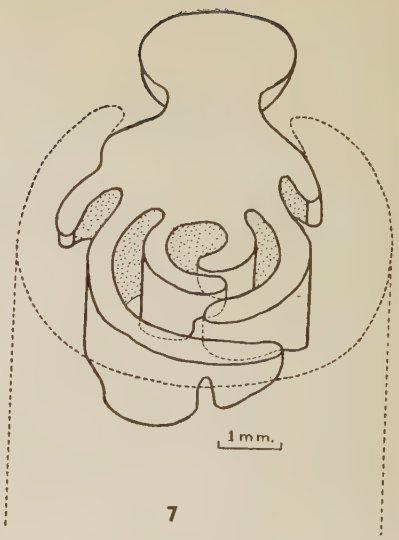
250μ

4

FIGS. 1-4 — Fig. 1. Seedling grown on damp cotton wool (*e*, endosperm; *c*, cotyledons; *ho*, holdfast; *ha*, haustorium). Fig. 2. Median section through a seedling about to penetrate the host (*e*, endosperm; *c*, cotyledons; *l*, first two leaves; *hy*, hypocotyl; *r*, radicle; *ho*, holdfast; *z*, zones of collapsed cells; *ha*, haustorium). Stippled areas indicate meristematic tissue, cross hatching vascular tissue. Fig. 3. Diagrammatic representation of the primary xylem of a seedling. The protoxylem is in solid black. The hypocotyl shows four bundles, in the short radicle region a solid cylinder of tetrarch xylem, in the holdfast two endarch xylem strands. Fig. 4 Section of host xylem (left) and advancing haustorium (stippled). The actively digesting cells of the haustorium appear to be those in close contact with host cells. Other parts of the haustorium are separated from the host by a deposit of red-staining gum (solid black).

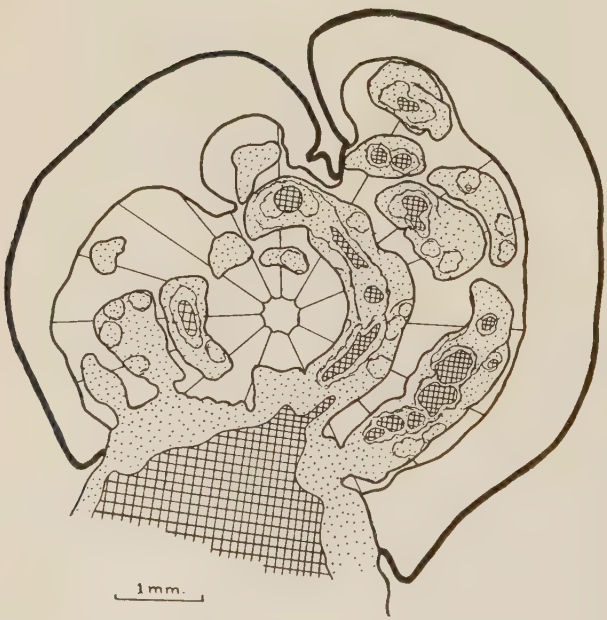


5a



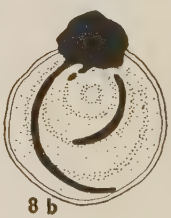
7

5b



6

8a



8b

FIGS. 5-8 — 5*a*. Section through swollen junction of host (*Pittosporum tenuifolium*) and parasite (stippled). *b*. Section of normal stem for comparison. Host phloem not shown. Fig. 6. Cross-section of an old host-parasite junction. Host: *Pittosporum tenuifolium*. Haustoria stippled and cross hatched to show parenchyma and vascular tissue respectively. Broken lines represent zones of collapsed cells round cortical meristems and vascular tissue of haustorium. FIG. 7. Part of a haustorial system reconstructed from serial sections. Host: *Pittosporum tenuifolium*. Fig. 8. Diagram of haustoria of approximately the same age in different host, *a* in *Melicytus ramiflorus*, *b* in *Pittosporum tenuifolium*. Parasite is solid black, the host dotted to represent annual rings.

approximately only half as long (Fig. 8). As a consequence of their slower rate of growth, the haustoria in *Pittosporum* grew outwards at a more acute angle. The shortest and most irregular were found in *Rubus australis*, the wood of which is extremely tough. *Rubus* stems attacked by the parasite became so greatly distorted at an early stage that the age of the haustoria could not be determined.

Anatomy of the Haustorium — As described above, growth of the haustorium within the host xylem is brought about by the combined activities of a succession of " growing points " developed at various places on the advancing front of the haustorium. The first sign of the development of a new " growing point " is the rapid division of a group of cells in the cortex of the haustorium. By further divisions and elongation of these cells a fresh protuberance invades the host xylem, usually spreading out further in the longitudinal direction than in the transverse. Zones of collapsed cells appear between the protuberance and the " parent " haustorium.

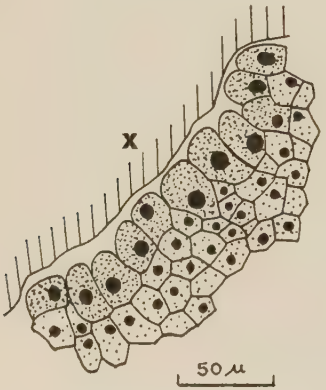
The epidermis of the primary haustorium was described as being composed of papillate or even hair-like cells, at least before penetration of the host. The epidermal cells of actively growing parts of the haustorial branches within the xylem are generally more rounded or pentagonal in outline (Fig. 4). This difference appears to be caused by compression within the host. Fig. 9 shows an actively growing haustorium which has pulled away from the host xylem: the cells are much more elongated. All such epidermal cells have dense contents and large, sometimes almost amoeboid nuclei, a characteristic of secretory tissue, and are evidently responsible for the secretion of enzymes which dissolve the opposing host tissue. The xylem of the host in contact with these secretory cells shows partially digested vessels and parts of fibres. In older, presumably non-growing parts of the haustoria, the epidermis is composed of more rounded, vacuolated cells and between them and the host is a layer of brownish material which stains bright red with safranin (Figs. 4, 10). It does not stain with Sudan III or with lignin stains so is neither cuticular in nature nor derived from the partial digestion of host xylem. It closely resembles a red-staining material often found in the vessels of the host near the haustorium (Fig. 10). The fact that it is sometimes found in droplets suggests that it is a mucilage or a gum — perhaps a gum produced by the host in response to wounding. In some cases so much gum was present that the haustoria appeared to be floating in it (Fig. 10).

The cortex is composed of closely packed, thin-walled parenchyma. These cells never become rounded off, probably because expansion is almost impossible within the host xylem. Many of them show signs of recent division parallel to the surface of the haustorium. A strange feature is the complete lack of stone cells and tannin which are so conspicuous in aerial parts of the plant. No periderm ever develops.

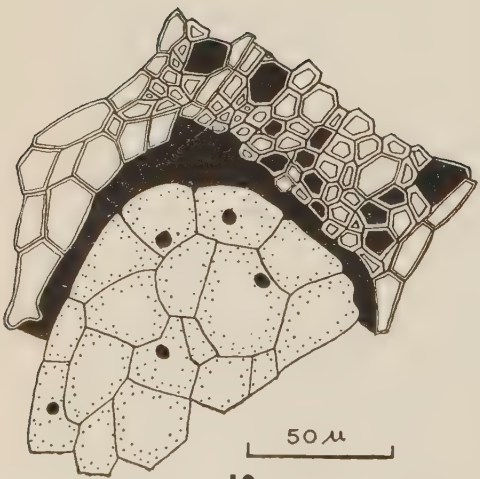
The middle of the haustorial strand is occupied by one or more isolated, collateral, vascular bundles. Differentiation of the vascular tissues takes place at a short distance behind a " growing point ". The first cells to become mature are short spiral or reticulate tracheids and/or vessels. A few indistinct phloem elements lie adjacent to them. A cambium soon develops between the xylem and phloem. It functions normally, cutting off xylem to one side and phloem to the other. Before the phloem cells can reach maturity, they are crushed and merge with the collapsed zones formed round each bundle during the initial burst of growth. Secondary thickening appears to be confined to the bundles.

The number and arrangement of the bundles varies in different parts of the haustorial system. Sometimes they are arranged more or less in a ring, giving a very stem-like appearance to the haustorium. In older haustoria they tend to be squashed up together, rather as if a former ring of bundles had suffered compression. Although the irregular growth of the haustorium makes it difficult to follow the stages in development of the vascular system, it would appear that each bundle is the " trace " appertaining to each new " growing point ".

In the cortex of the haustorium, spherical groups of meristematic cells appear. The division and expansion of these cells exerts a pressure which results in the collapse of adjacent, non-meristematic cortical cells. (Plate II, Fig. 7). Such cortical meristems abut directly on to the host xylem; at this point a number of elongated, hair-like cells with dense contents and large nuclei are developed. One of these grows out into the host xylem, often for a considerable distance and eventually applies itself to the wall of a host vessel or tracheid (Plate II, Fig. 8). Meanwhile differentiation of short reticulate tracheids and vessels proceeds gradually outwards from the main vascular tissue of the haustorium into these meristems. Finally the long absorbing cell is converted into a vessel (Plate II, Fig. 8), thus providing a direct, water-conducting channel from host to parasite. Many such cortical meristems, each surrounded by a zone of collapsed cells, can be seen in Fig. 6.



9



10

Figs. 9, 10 — Fig. 9. Section of an haustorium (stippled) which has pulled away from the host xylem (*x*). The epidermal cells of the haustorium are papillate. Fig. 10. Section of an haustorium no longer growing actively and separated from the host xylem by a thick layer of brown gum. Similar material is present in the vessels and fibres of the host adjacent to the haustorium.

The Path of Travel of the Haustorium — In the majority of unions of host and parasite investigated, there is differentiated or partly differentiated host xylem outside all parts of the youngest haustorium. Travel of the haustorium must therefore be entirely within the host xylem. There is nothing to indicate that these haustoria had ceased to grow and had been buried by later formed host xylem. Regions could be found with papillate epidermal cells and no gum, indicating active growth.

Some haustoria have been found, however, with an anatomical structure very different from that described above. It seems that these differences are bound up with an unusual path of travel, i.e. travel outside the xylem cylinder. In one or two young plants, the primary haustorium was found in the cambial zone and had apparently split the xylem away from the phloem. The haustorium had few collapsed zones and no cortical meristems and xylem connections. In haustoria growing in leaves, there are peculiar spherical spaces in the cortex which might be bursts of growth which had died out at an early stage, or, alternatively, cortical meristems which had failed to develop. Such holes are also found in haustoria which grow out of the xylem of the host into the phloem or cortex. This has been seen in large galls where the host xylem is very irregular. The haustorium has not been able to follow the convolutions of the xylem cylinder and has grown straight on. In these haustoria, all stages of disintegration

of a cortical meristem to a hole can be found. There are no lines of collapsed cells and usually greater differentiation of phloem. This evidence supports the hypothesis that many of the features of normal haustoria are a result of their compression within the host xylem.

The fact that stone cells and tannin are not found in the cortex of haustoria, although they occur in all other parts is also connected in some way with the haustoria being within the host xylem. One haustorium of a large plant had come to the surface of the host either by growing into a crack in the stem or by being exposed when a crack developed. The haustorium was apparently still alive, and the cortical cells of the exposed part contained tannin, were rounded off and some had been converted into stone cells. There were no lines of collapsed cells.

Secondary Haustoria from Aerial Roots — Haustoria may be produced from aerial roots at any stage of development. The first external sign is a light-coloured swelling on the side of the root pressed against either the host or another root. The haustorium arises endogenously between two protoxylem "points". In most respects these secondary haustoria behave like the primary ones. Anatomically they differ mainly in that there are usually more vascular bundles (7-9) and these are arranged in an arc. When shoots arise from roots, they usually appear near haustoria.

Self-parasitism — *L. micranthus* is occasionally the victim of self-parasitism. Roots often send haustoria into other roots, but such self-parasitic roots have never been seen to produce shoots. A microscopical examination of self-parasitizing haustoria showed several differences from normal. Instead of confining themselves to the outer region of the xylem, they destroy the cambium and part of the phloem as well. The characteristic zones of collapsed cells round both the vascular tissue and the xylem connections are absent and there is greater phloem development. Stone cells are also abundant. At the tip of such an haustorium there appears to be union between both the xylem and the phloem elements of the "host" and "parasite". This self-parasitic relationship is thus very different from that in "foreign" hosts and more closely resembles a graft.

Discussion

A considerable amount of descriptive work on the various types of haustorial development in the Loranthaceae has been carried out, particularly in Germany. Engler & Krause (1935) describe the haustoria under seven headings. The type found in *L. micranthus* agrees very closely with that described above as characteristic of certain members of the tropical genus *Struthanthus*, viz. *S. marginatus*, *S. deppeanus*, *S. vulgaris* and *S. quercicola*. The haustoria of these species have been very fully described by Heil (1926). He found that secondary haustoria arose from creeping roots and penetrated host stems (or other roots of the parasite). When the xylem of the host was reached, further radial growth of the haustorium ceased; tongue-like branches – "Saugfortsatze"— were then produced which travelled tangentially round the stem in the region of the cambium. Spherical groups of meristematic cells — the so-called "Haustorialkerne" — arose in the cortex, associated with zones of collapsed cells—"Demarkationslinie"— as in *Loranthus micranthus*; where these abutted on to the host xylem, the actual water-absorbing organs—"saugfaden" or Haarzellen"—grew out. Heil describes one specimen of *Struthanthus quercicola* in which the earlier formed haustorial tongues had become embedded in secondary xylem and new tongues developed outside of them. The resemblance between this plant and *Loranthus micranthus* is very striking. Moreover, similarities between the species extend to parts other than the haustoria. The anatomical structure of the creeping roots, for example, is so alike in the two that the longitudinal section of a root tip figured by Heil could be substituted for that of *L. micranthus*.

There has been considerable controversy as to the phylogeny of the haustoria in the Loranthaceae, especially in the group in which cortical strands are produced. At a first glance, there seems

little doubt that the haustoria of *L. micranthus* are of the nature of modified roots: the primary ones develop directly from the radicle and the secondary ones arise endogenously from the secondary roots in the manner of lateral roots. A closer investigation, especially into stelar anatomy, reveals characters which are far from typical of roots. The occurrence in a root-like organ of isolated collateral bundles to which secondary growth is confined is certainly remarkable. Haberlandt (1914) points out that they occur in lateral roots that have been modified to form tuberous organs in such plants as *Dioscorea batatas, Sedum telephium* and the *Ophrydeae.* He quotes Van Tieghem as saying that such "bundles" really represent central cylinders and that the tuber owes its origin to the (phylogenetic) fusion of several distinct lateral roots. It is not impossible to imagine a similar origin for the somewhat tuberous haustoria of *Loranthus micranthus,* especially in view of the restricted conditions forced upon them within the host xylem. Heil (1926) certainly seems to favour the view that haustoria are of the nature of roots which have been profoundly modified and progressively reduced. Hence he believes that the cortical meristems with their associated absorbing threads are second order lateral roots, particularly on the grounds of their more or less endogenous origin.

One cannot help but feel that a certain amount of caution should be exercised in comparing an extremely highly specialized structure like an haustorium with a normal root. There is much to be said for the view of Thoday (1951) that "whether or not it (the haustorium) arose as an organ *sui generis,* its unique character requires that it should be so regarded now".

Summary

The primary haustorium of *Loranthus micranthus* is derived from the swollen tip of the hypocotyl of the seedling. The haustorium grows straight in through the host stem until it reaches the xylem. Branches are then produced which grow in a tangential direction in the young xylem, moving gradually outwards to keep pace with the formation of new xylem. One haustorial branch appears to grow actively for two or three years; further branches are then produced from the main axis at successive outer levels in the host xylem.

The haustorium penetrates the host partly by pressure and partly by enzyme action. The epidermal cells of actively growing parts of haustoria show secretory characters and are evidently responsible for secretion of enzymes.

Haustorial branches are flange-like structures with an irregular outline resulting from their sporadic type of growth. The vascular tissues consist of a number of collateral vascular bundles to which secondary growth is confined.

The water-absorbing elements of the haustoria are solitary, elongated cells which develop from groups of meristematic cells in the cortex, grow out into the host xylem and apply themselves to the walls of vessels or tracheids. They are later differentiated into vessels which connect the vascular tissues of host with parasite.

All parts of the haustoria are characterized by peculiar zones of collapsed cells which appear to result from internal pressures set up inside the haustorium by meristematic activity which is resisted by the rigid host xylem.

This paper forms part of a thesis submitted to the University of New Zealand for the degree of Master of Science. I wish to acknowledge my thanks to Dr. L. H. Millener, who suggested the problem, and to Professor V. J. Chapman and Dr. G. K. Sutherland for advice during the course of the work. I am also indebted to Professor D. Thoday for his very helpful criticism.

Postscript — Since this paper went to press, my attention has been drawn to a recently published account of *Dendrophthoe falcata* (L.f.) Ettings. (Singh, 1954), in which the vascular tissue of the holdfast and haustorium, like that of *Loranthus micranthus,* consists of a ring of open, endarch, collateral bundles. It seems possible that this stem-like appearance of haustoria may prove, on further investigation, to be common in the Loranthaceae.

Literature Cited

CHEESEMAN, T. F. 1925. "Manual of the New Zealand Flora." Wellington.

ENGLER, A. & KRAUSE, K. 1935. Loranthaceae. In "Die natürlichen Pflanzenfamilien." Auf. 2, 16b.

HABERLANDT, G. 1914. "Physiological Plant Anatomy." London.

HEIL, H. 1926. Haustorialstudien an *Struthanthus*-Arten. Flora **21**: 40-79.

KÜSTER, E. 1930. Anatomie der Gallen. In K. Linsbauer, "Handbuch der Pflanzenanatomie." Abt, 1, Teil **3**: Band V/1.

LAING. R. M. & BLACKWELL, E. W. 1940. "Plants of New Zealand." New Zealand.

SINGH, B. 1954. Studies in the family Loranthaceae — list of new hosts of *Dendrophthoe falcata* (L.f.) Ettings., its relations with hosts, the anatomy of its seedling and mature haustorium. Agra Univ. J. Res. **3**: 301-315.

THODAY, D. 1951. The haustorial system of *Viscum album*. J. exp. Bot. **2**: 1-19.

FLORAL MORPHOLOGY AND EMBRYOLOGY OF *FUMARIA PARVIFLORA* LAMK.

H. B. SAKSENA

Victoria College, Gwalior, India

Introduction

Schnarf (1931) has reviewed the older literature on the family Papaveraceae which once included the genus *Fumaria*, now usually placed in a separate family Fumariaceae. So far there seems to be no detailed work on the gametophytes and seed development of *F. parviflora*. Souéges has studied the embryogeny of *Fumaria officinalis* (1941a, 1941b). The present study was undertaken at the suggestion of Professor P. Maheshwari of the Delhi University.

Fumaria parviflora is a light-green, much-branched, glaucous annual herb with watery latex. It appears in the month of November as a weed of cultivation, especially in wheat fields, and lasts up to March. The fruit is of medicinal value and is used in the treatment of fever and ague.

The material was fixed in formalin-acetic-alcohol from Gwalior during the years 1949-1951. The usual methods of infiltration and embedding were followed. Sections were cut 5-16 μ thick and stained in iron-haematoxylin and in safranin-fast green. The former gave better results.

Observations

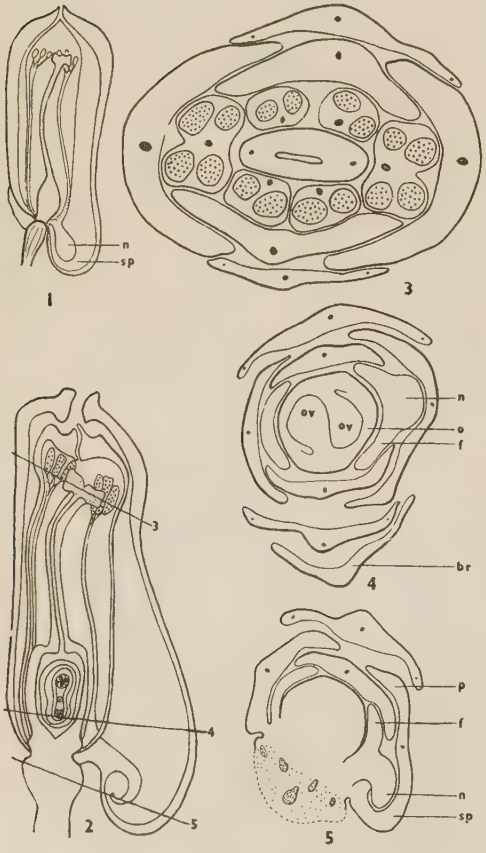
FLOWER — The floral organs develop in acropetal succession and the small bracteate flowers are produced in racemes, ½-1 in. long, which may be terminal or leaf-opposed. As the buds mature, the rosy-purplish colour of the petals becomes more prominent. A fully developed flower has 2 minute lanceolate deciduous sepals. The 4 petals are arranged in two whorls. The outer 2 petals are large, lateral and dissimilar, and almost enclose the flower; One shows a sac-like spur at the base. The 2 inner petals are narrow, erect, medianly placed, often coherent at the tip, and keeled at the back. There are 2 tripartite stamens situated opposite the outer petals. The central anther of each stamen is dithecous while the laterals are monothecous (Fig. 3). The basal part of the filament opposite the spur (*sp*)

develops a nectary gland (*n*) which protrudes into the spur (Figs. 1, 2, 5). The flower is thus laterally zygomorphic.

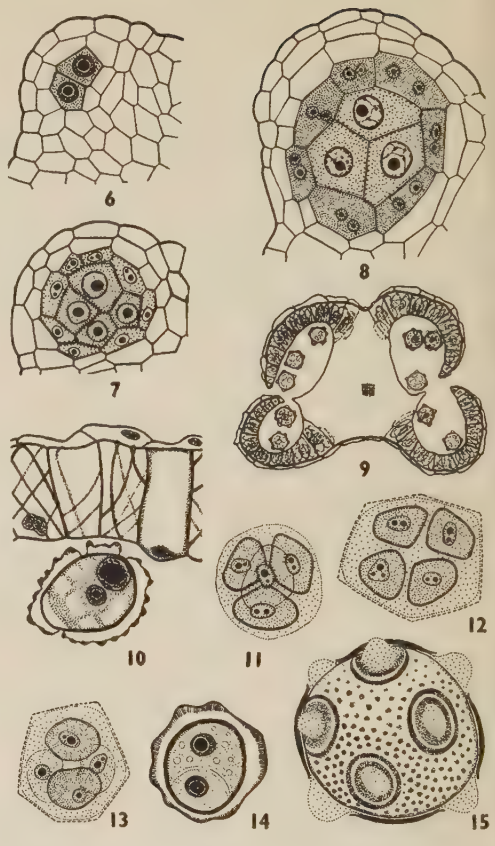
The origin of the nectary and the course of staminal bundles have already been investigated by Arber (1931) and Norris (1941) and will not be dealt with here.

The gynoecium shows a hollow style, bilobed stigma, and unilocular ovary with 2 ovules on parietal placentae (Figs. 2, 4).

The flowers are highly protandrous and the pollen grains are shed when the ovules



Figs. 1-5 — Floral morphology (*br*, bract; *f*, filament; *n*, nectary; *o*, ovary; *ov*, ovule; *p*, petal; *sp*, spur). Fig. 1. Bud showing disposition of nectary, androecium and gynoecium. × 15. Fig. 2. L.s. older bud (diagrammatic). × 40. Figs. 3-5. T.s. flower, approximately at levels marked 3, 4 and 5 in Fig. 2. Fig. 3. × 112. Figs. 4-5. × 40.



Figs. 6-15 — Microsporogenesis and male gametophyte. Figs. 6-8. T.s. anther lobes showing formation of wall layers and microspore mother cells. × 500. Fig. 9. T.s. dehisced anther. × 106. Fig. 10. Part of anther wall and a pollen grain at maturity. × 500. Figs. 11-13. Tetrahedral, iso-bilateral and decussate tetrads. × 696. Fig. 14. Bi-celled pollen grain. × 696. Fig. 15. Pollen grain showing sculpturing of exine. × 696.

are still at the functioning megaspore stage. One of the ovules aborts, and the fruit is a single-seeded, indehiscent, hard, globose nutlet. The seeds are albuminous.

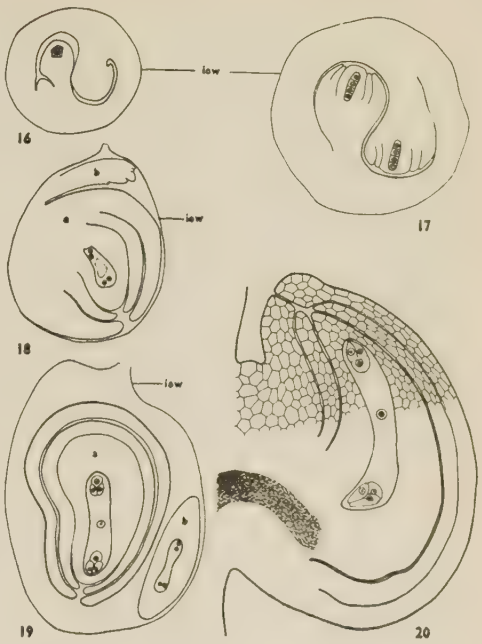
The nature of the androecium of *Fumaria* is controversial. According to Hooker (1872), Duthie (1903), Johnson (1930) and Lawrence (1951), there are 6 diadelphous stamens, while Rendle (1952) considers that there are only 2 tripartite stamens. On the basis of an anatomical study, Arber (1931) has concluded that there are 6 stamens — 2 normal and

dithecal, and 4 others reduced and monothecal, yet independent members and not 'half' stamens. On the other hand, Saunders (1928, 1932) considers that there are 2 whorls of 2 stamens in each of which the 2 median ones have bifurcated. This hypothesis, however, is not substantiated by the course of vascular bundles (*see* Arber, 1931). According to Eichler (1875-78) and Gray (see Rendle, 1952) there are only 2 lateral stamens.

MICROSPOROGENESIS — Simultaneous with the appearance of the anther lobes, hypodermal archesporial cells become distinguishable in each lobe. These divide periclinally to form the primary parietal layer and the sporogenous tissue (Fig. 6). The former divides again to give rise to an outer layer — the endothecium, and an inner layer which by another periclinal division differentiates into the middle layer and the tapetum (Figs. 7, 8). The tapetum is of the glandular type and its cells become binucleate before the microspore mother cells enter the reduction divisions (Fig. 8). The enlargement of the tapetum crushes the middle layer which is scarcely recognizable at the close of the reduction divisions. At about this time the tapetum also begins to show signs of degeneration and is already disorganized at the 2-celled stage of the pollen grains. The endothecium develops the usual fibrous thickenings and at maturity the wall of the anther consists of only this layer and the compressed epidermis (Figs. 9, 10).

The microspore mother cells undergo simultaneous reduction divisions and cytokinesis takes place by furrowing. The tetrads are usually tetrahedral but isobilateral and decussate arrangements are not infrequent (Figs. 11-13). During reduction divisions, a special mucilaginous wall is secreted between the original wall and the protoplast of the microspore mother cell. It becomes prominent during tetrad formation but is consumed when the microspores enlarge and separate from one another.

MALE GAMETOPHYTE — The microspore nucleus divides to produce a lenticular generative and a large vegetative cell separated by an ephemeral membrane (Fig. 14). The latter soon begins to disorganize and the generative cell moves up into the general cytoplasm of the pollen grain. No further division occurs and the pollen grains are shed at this stage (Fig.10). They are acolpate and forminate[1] with a broad nexine[1], thick verrucate sexine[1] and eight bulging germ pores (Fig. 15).



FIGS. 16-20 — Development of ovule (*iow*, inner ovary wall). Figs. 16-17. T.s. ovary with 2 young ovules (reconstructed). × 92. Figs. 18,19. L.s. ovary with functional *a* and abortive *b* ovules (reconstructed). × 92. Fig. 20. L.s. ovule at mature embryo sac stage (reconstructed). × 150.

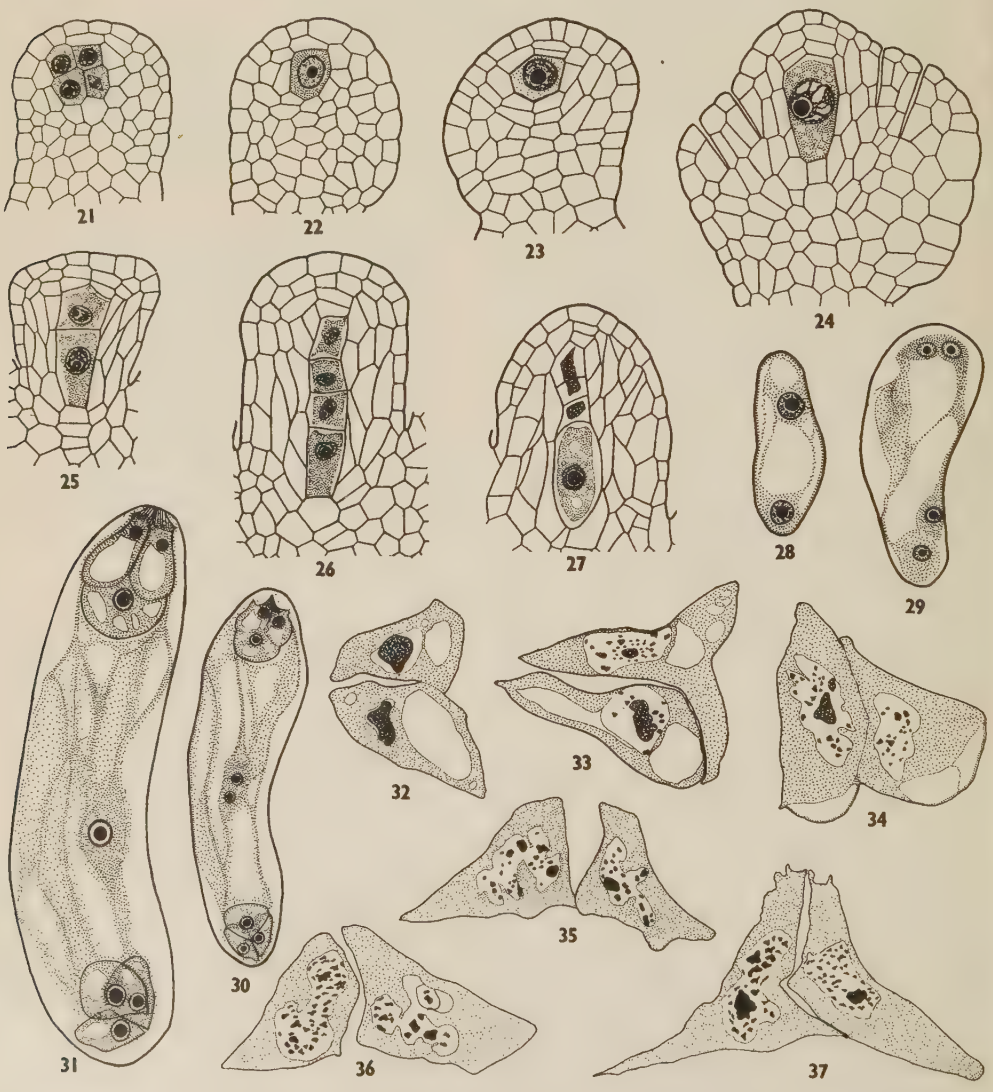
OVULE — The ovules are campylotropous, bitegmic and crassinucellate. When they arise, the anthers are at the microspore mother cell stage. Both the ovules in an ovary develop concurrently for a time (Figs. 16, 17) but after megaspore formation one aborts (Figs. 18, 19). Only in one case the abortive ovule (*b*) showed a 4-nucleate embryo sac while the functional ovule (*a*) had an 8-nucleate gametophyte (Fig. 19). A slight curvature of the ovule is noticeable even at the 4-nucleate stage. This becomes more pronounced at maturity (Figs. 18, 20).

1. Terminology according to Erdtman (1952).

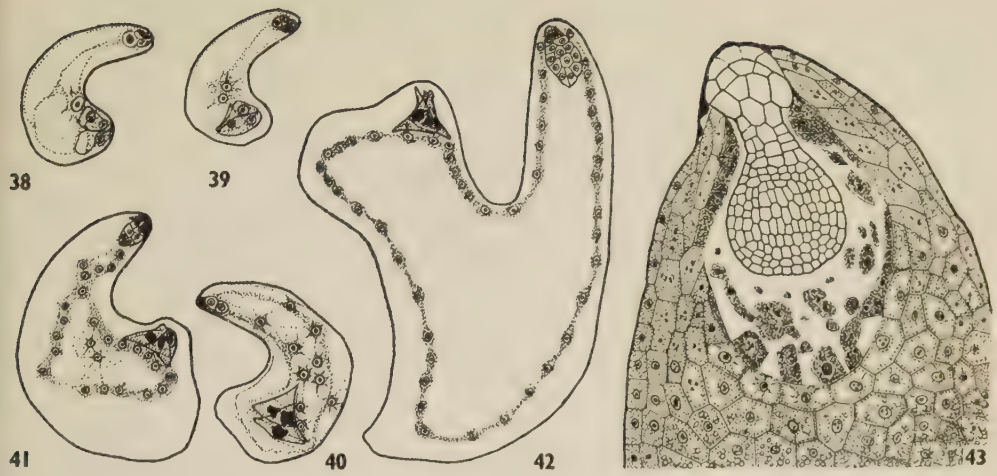
The funicular vascular strand terminates at the chalaza.

The integuments make their appearance at the megaspore mother cell stage (Fig. 24), and both take part in the formation of the narrow micropyle. Each is 3-layered for the most part but the outer is thicker at the apex while the inner is only 2-layered in this region (Fig. 20). After fertilization the integuments get closely appressed to each other.

Megasporogenesis — Usually one, but sometimes two or more hypodermal archesporial cells differentiate in the young nucellus (Figs. 21, 22). The first periclinal division results in the formation



Figs. 21-37 — Megasporogenesis and female gametophyte. Fig. 21. L.s. nucellus with a group of 4 sporogenous cells. × 440. Figs. 22-24. Same, showing megaspore mother cell and parietal cells. × 440. Figs. 25,26. Dyad and tetrad of megaspores. × 440. Fig. 27. Functioning megaspore. × 440. Figs. 28-30. 2-, 4- and 8-nucleate embryo sacs. ×1440. Fig. 31. Organized mature embryo sac, polars have fused. × 440. Figs. 32-37. Hypertrophied condition of antipodal cells, the nuclei are lobed and the chromatin has broken up into fragments. × 202.



FIGS. 38-43 — Development of endosperm. Fig. 38. Fertilized embryo sac. × 50. Fig. 39. Embryo sac with 2-celled proembryo and 2 free endosperm nuclei. × 50. Figs. 40-42. Same, advanced stages. × 50. Fig. 43. Upper part of embryo sac showing advanced proembryo and cellular endosperm, entire ovule from which this figure has been enlarged is shown in Fig. 69. × 139.

of a parietal cell and a megaspore mother cell. The former undergoes one or two further divisions so that the megaspore mother cell is separated from the nucellar epidermis by a couple of layers (Figs. 23, 24). It undergoes the usual meiotic divisions to form an axial row of four megaspores (Figs. 25, 26). The chalazal megaspore enlarges while the upper three degenerate (Fig. 27).

EMBRYO SAC — The functional megaspore divides giving rise to 2 nuclei which undergo two further divisions leading to the 4- and 8-nucleate embryo sacs (Figs. 28-30). The synergids show a large vacuole in the broad basal part and a nucleus and filiform apparatus in the upper. The egg protrudes below the synergids (Fig. 31). The two polar nuclei fuse quite early and the fusion nucleus lies almost in the middle of the embryo sac.

The uninucleate, large and vacuolated antipodal cells simulate the egg apparatus. This condition also occurs in *Caltha* (Grafl, 1941) and *Argemone* (Sachar, 1953) and several other plants (see Maheshwari, 1950).

Soon after the fusion of the polar nuclei, the antipodal cells enlarge and become greatly hypertrophied, particularly during the post-fertilization stages. With the increase in the size of the cell the nucleus also enlarges and becomes lobed. The chromatic material seems to break up and its fragments are distributed irregularly within the nuclear membrane (Figs. 32-37). It is only when the embryo is well advanced and the cotyledons have been initiated that the antipodals are absorbed.

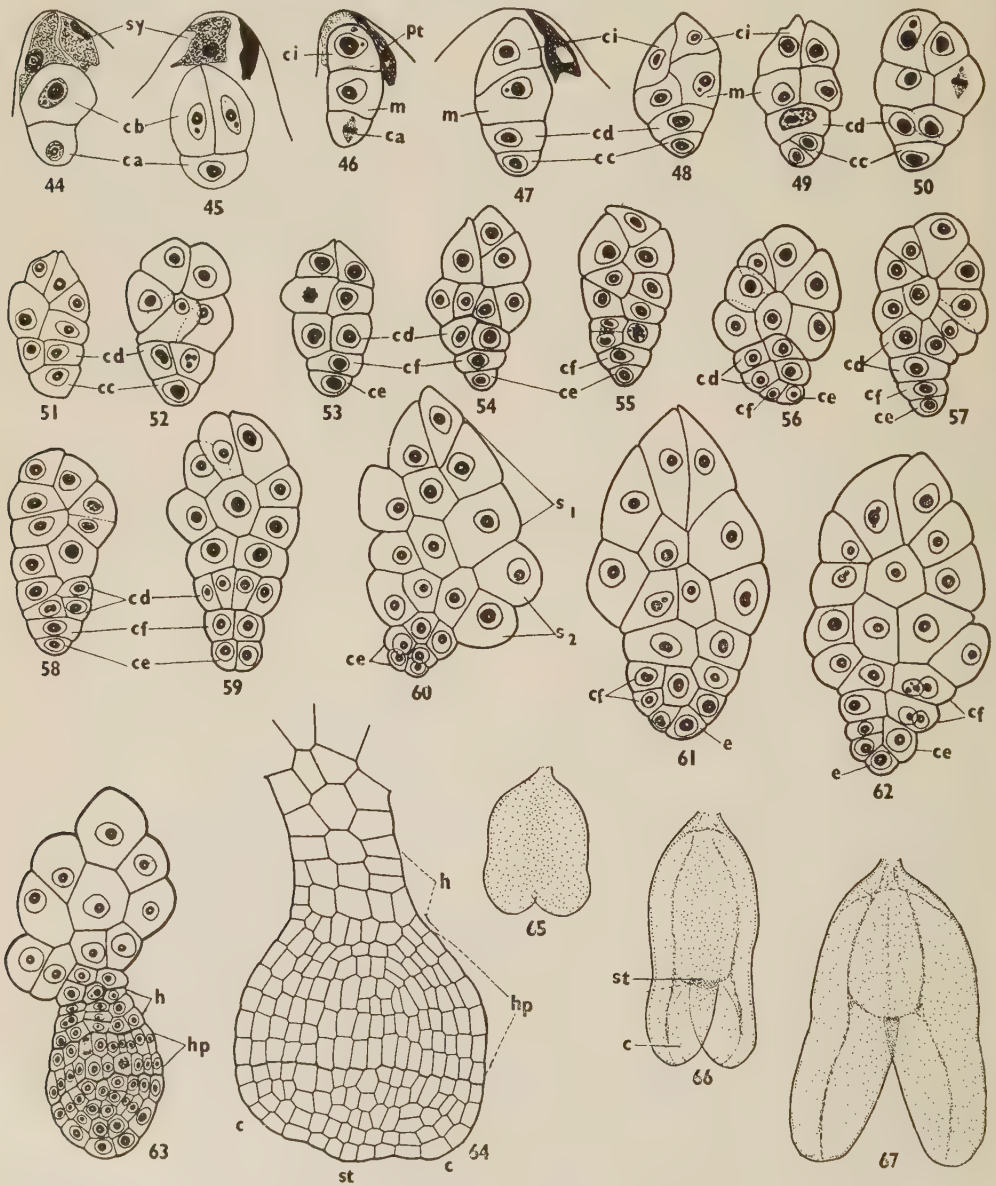
Huss (1906) has already called attention to the enlargement of the antipodals of *Fumaria*, *Corydalis* and *Papaver*. This is apparently a characteristic feature of the family Papaveraceae (Schürhoff, 1926) and has also been reported in *Argemone mexicana* (Joshi, 1933; Bose, 1937; Sachar, 1953). Grafl (1941) mentions that in *Caltha palustris* they attain a high degree of polyploidy and offer a close analogy with the behaviour of the cells of the anther tapetum (see Maheshwari, 1950).

POLLINATION AND FERTILIZATION — The highly protandrous condition of the flower and the presence of a well-developed nectary and spur suggest insect pollination. Self-pollination was, however, observed in some closed buds and pollen grains were seen germinating on the stigma.

Double fertilization was not observed but remnants of the pollen tube in post-fertilized embryo sacs indicate that probably it occurs normally. The synergids show signs of degeneration soon after

fertilization and by the time the proembryo is 8- to 10-celled they are completely disorganized.

ENDOSPERM — The primary endosperm nucleus lies adjacent to the antipodal group and divides at about the same time as the oospore (Figs. 38, 39). Subsequent divisions take place rapidly and when the proembryo is still 2-celled, there are 2-10 endosperm nuclei (Figs. 39, 40). With further divisions, the nuclei, of which several hundred may be counted, take up



FIGS. 44-67 — Development of embryo (*c*, cotyledon; *e*, epiphysis; *h*, hypophysis; *hp*, hypocotyl; *pt*, pollen tube; *sy*, synergid; s_1, s_2, lower and upper part of the suspensor respectively; *st*, stem tip). For explanation, see text. Figs. 44-64. × 292. Figs. 65-67. From whole mounts of embryos. × 46.

a peripheral position and are embedded in dense cytoplasm (Figs. 41, 42). Wall formation is centripetal and gradually fills the entire embryo sac. To begin with, the endosperm cells are thin-walled and show characteristic cytoplasmic strands (Fig. 43). During maturation of the seed the walls thicken and the cells become filled with starch (Fig. 74).

EMBRYO — The embryogeny resembles that of *Fumaria officinalis* as described by Souèges (1941a, 1941b).

The oospore enlarges and becomes vacuolated. The first division is transverse and gives rise to an apical cell *ca* and a basal cell *cb* which divide again by transverse walls producing the cells *cc* and *cd*, and *m* and *ci* respectively (Figs. 44, 46, 47). In one case the basal cell *cb* had divided by a vertical wall (Fig. 45). Subsequent divisions of *ci* and *m* are variable and ultimately produce the lower part of a massive suspensor (Figs. 48-59) whose cells are large and highly vacuolate. The subterminal cell *cd*, a derivative of *ca*, divides by vertical and transverse walls into two tiers of cells giving rise to the upper part of the suspensor (Figs. 57-62). The suspensor cells enlarge and assume a characteristic shape looking like a bunch of grapes (Figs. 60-62).

The terminal cell *cc* divides by a transverse wall into *ce* and *cf* (Figs. 53-55, 57, 58). The latter divides further by vertical and transverse walls producing two tiers (Figs. 61,62). The lower tier adjacent to the suspensor forms the hypophysis (*h*) and the upper tier gives rise to the hypocotyl (*hp*) (Figs. 63, 64). The cell *ce* usually divides by an oblique wall (Fig. 60) and its daughter cells divide again resulting in a tetrahedron of which one cell *e*, occupying the apex (Figs. 61, 62), differentiates into the epiphysis. It gives rise to the plumule. The three basal cells of the tetrahedron produce the cotyledons (Figs. 63, 64, upper part of the suspensor has been left out in the latter figure). The hypocotyl as well as the cotyledons soon elongate in size (Figs. 65-67).

Thus the cells *cb* and *cd* form only the suspensor while the cell *cc* gives rise to the embryo proper.

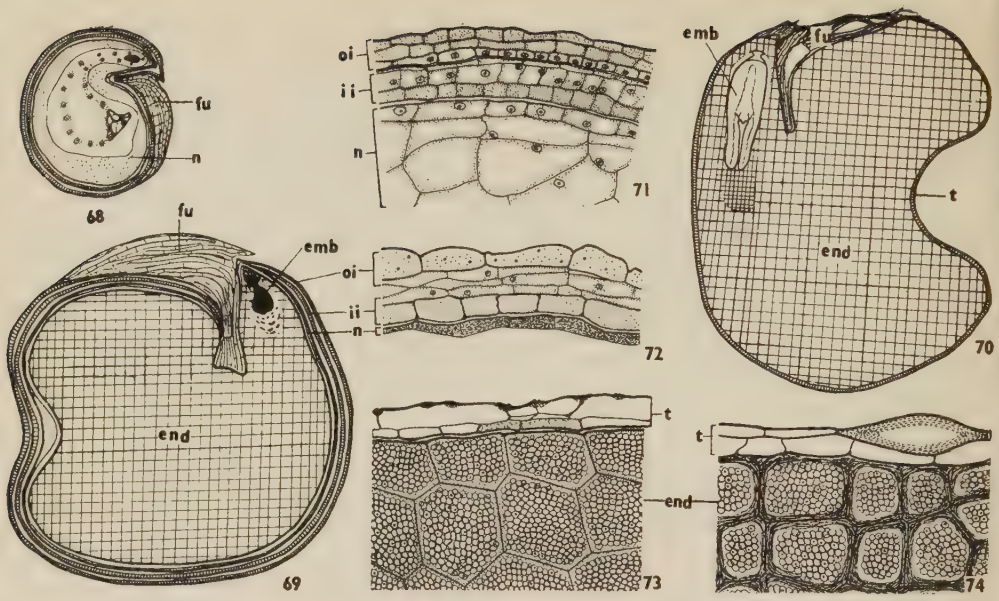
In short, the embryogeny of *Fumaria parviflora* resembles that of *F. officinalis* (Souèges, 1941a, 1941b) and falls under the Megarche type 6 of the second big division in the 13th embryogenic group.

SEED AND FRUIT — The nucellus is completely absorbed during the maturation of the seed (Figs. 68-74). At the same time, the two inner layers of the outer integument and the two outer layers of the inner integument are also crushed and flattened. The walls of the innermost layer of the inner and the outermost layer of the outer integument become thickened and form the testa. Cells of the inner layer also develop pitted thickenings (Fig. 73).

The ovary wall consists of many layers of cells. After fertilization it shows three distinct regions — inner, middle and outer. The cells of the middle region develop prominent sclerotic thickenings from inside outwards. This portion forms the hard wall of the nut. The inner and outer regions of the ovary wall are compressed and crushed due to enlargement of the middle region. Remnants of the inner compressed layers form a leathery lining on the inner side of the pericarp and those of the outer region peel off here and there giving a rugose appearance to the ripe fruit.

SYSTEMATIC POSITION — The assignment of a family rank to the Fumariaceae has been somewhat controversial. Duthie (1903) includes *Fumaria* within the family Papaveraceae. Hutchinson (1926) gives Fumariaceae a family rank but includes the sub-family Hypecoideae under it. Engler & Diels (1936) and Rendle (1952) treat Fumarioideae as a sub-family of Papaveraceae along with the two other sub-families — Hypecoideae and Papaveroideae. Erdtman (1952) regards the pollen type of *Fumaria* as more or less unique and considerably different from that in the other members of the Fumarioideae so far investigated, but is reluctant to give this tribe a family rank.

However, the absence of latex, the almost closed nature of the flowers which are in striking contrast with the wide open flowers of the Papaveraceae, their lateral zygomorphy, the presence of a sac-like spur, two lateral tripartite stamens and the sequence of embryogeny seem to justify the erection of the family Fumariaceae (see also Lawrence, 1951).



FIGS. 68-74 — Development of seed (*emb*, embryo; *end*, endosperm; *fu*, funiculus; *ii*, inner integument; *n*, nucellus; *oi*, outer integument; *t*, testa). Figs. 68-70. L.s. ovules showing progressive development of endosperm, embryo and seed coat (diagrammatic). × 27. Figs. 71-73. Enlarged portions of Figs. 68-70 respectively. × 264. Fig. 74. Part of testa and endosperm from almost mature seed. × 264.

Summary

1. The flower shows a lateral zygomorphy. One of the lateral petals is spurred and lodges the nectary formed from the basal part of the adjacent filament.

2. There are two tripartite stamens. The middle member of each stamen shows four pollen sacs while the two lateral ones have only two pollen sacs each. The anther wall consists of the epidermis, fibrous endothecium, a middle layer and the glandular binucleate tapetum.

3. The flowers are strongly protandrous. The reduction divisions are simultaneous, cytokinesis occurs by furrowing and the tetrads are commonly tetrahedral. Isobilateral, and decussate tetrads are also found sometimes.

4. The pollen grains are acolpate and bicelled. There is a conspicuous intine, a thick exine, and 8 bulging germ pores.

5. Two bitegmic, crassinucellate, campylotropous ovules are present, but only one of them matures into a seed.

6. The development of the embryo sac is of the " Polygonum type ". The three antipodals simulate the egg apparatus and become greatly hypertrophied in post-fertilization stages. Their nuclei enlarge, become amoeboid, and the chromatic material breaks up into fragments which are distributed irregularly within the nuclear membrane.

7. The endosperm is Nuclear, wall formation occurs at a late stage and the cells are full of starch.

8. The oospore forms a 4-celled filamentous proembryo, three cells of which enter into the formation of the massive suspensor and the terminal cell alone produces the embryo proper.

9. The seed is albuminous and shows a typical dicotyledonous embryo. The nucellus is consumed and only the outermost layer of the outer integument and the innermost layer of the inner integument form the seed coat.

I have great pleasure in expressing my gratitude to Professor P. Maheshwari and

Dr. B. M. Johri, University of Delhi, for their kind help and guidance in the preparation of this paper; to Professor R. Souèges, University of Paris, for his valuable suggestions regarding the development of the embryo; and to Professor K. L. Saksena, Victoria College, Gwalior, for encouragement.

Literature Cited

ARBER, A. 1931. Studies in floral morphology. III. On the Fumarioideae with special reference to the androecium. New Phytol. **30**: 317-354.

BOSE, P. K. 1937. Cytological studies of *Argemone*. J. Indian Bot. Soc. **16**: 197-208.

DUTHIE, J. F. 1903. "Flora of the Upper Gangetic Plain." Calcutta.

*EICHLER, A. W. 1875-1878. "Blüthendiagramme."

ENGLER, A. & DIELS, L. 1936. "Syllabus der Pflanzenfamilien." Berlin.

ERDTMAN, G. 1952. "Pollen Morphology and Plant Taxonomy." Waltham, Mass., U.S.A.

*GRAFL, I. 1941. Über das Wachstum der Antipodenkerne von *Caltha palustris*. Chromosoma **2**: 1-11.

HOOKER, J. D. 1872. "Flora of British India." London.

*HUSS, G. A. 1906. Beiträge zur Morphologie und Physiologie der Antipoden. Bieh. bot. Zbl. **20**: 77-174.

HUTCHINSON, J. 1926. "The Families of Flowering Plants." Vol .I — Dicotyledons. London.

JOHNSON, A. M. 1930. "Taxonomy of the Flowering Plants." New York.

JOSHI, A. C. 1933. Megaspore formation and embryo sac of *Argemone mexicana*. J. Indian Bot. Soc. **12**: 83-91.

*Not seen in original.

LAWRENCE, G. H. M. 1951. "Taxonomy of Vascular Plants." New York.

MAHESHWARI, P. 1950. "An Introduction to the Embryology of Angiosperms." New York.

*NORRIS, T. 1941. Torus anatomy and nectary characteristics as phylogenetic criteria in the Rhoeadales. American J. Bot. **28**: 101-113.

RENDLE, A. B. 1952. "The Classification of Flowering Plants." Vol. II — Dicotyledons. Cambridge.

SACHAR, R. C. 1953. A reinvestigation of the embryology of *Argemone mexicana*. Curr. Sci. **22**: 381-383.

SAUNDERS, E. R. 1928. Illustrations of carpel polymorphism II. New Phytol. **27**: 175-192.

— 1932. On some recent contributions and criticisms dealing with morphology in angiosperms. New Phytol. **31**: 174-219.

SCHNARF, K. 1931. "Vergleichende Embryologie der Angiospermen." Berlin.

SCHÜRHOFF, P. N. 1926. "Die Zytologie der Blütenpflanzen." Stuttgart.

SOUÈGES, R. 1941a. Embryogenie des Fumariacees. L'origine du corps de l'embryon chez le *Fumaria officinalis* L. C. R. Acad. Sci. (Paris) **213**: 528-530.

— 1941b. Embryogenie des Fumariacees. La differenciation des regions fondamentales du corps chez le *Fumaria officinalis* L. C.R. Acad. Sci. (Paris) **213**: 699-702.

THE DEVELOPMENT OF THE EMBRYO OF *CYAMOPSIS*, *DESMODIUM* AND *LESPEDEZA* WITH A DISCUSSION ON THE POSITION OF THE PAPILIONACEAE IN THE SYSTEM OF EMBRYOGENIC CLASSIFICATION*

M. ANANTASWAMY RAU
Department of Botany, Central College, Bangalore, India

In a review of recent work on embryogeny, Souèges & Crété (1952) have discussed the importance of the Papilionaceae in their embryogenic system of classification and also emphasized the need for further investigations on the family. The profound variations that are seen in the development of the embryo and the fact that the Papilionaceae are represented in several groups of the periodic system of classification have given this family added importance. The present author has already studied several members of the family (Anantaswamy Rau, 1950, 1951a, b) and during his recent visit to the U.S.A. opportunity was taken to study the embryogeny of the following: *Cyamopsis psoralioides* DC., belonging to the tribe Galegeae; and *Desmodium canadense* DC., *D. laevigatum* DC., *D. canescens* DC., and *Lespedeza violacea* Pers. of the tribe Hedysereae. A descriptive account of the development of the embryo in the above members and a brief discussion of the position of the Papilionaceae in the embryogenic system of classification are included in this paper.

The material of *Cyamopsis* was collected in Mysore City, India, and of the others in the Marion and Adams counties of the State of Ohio, U.S.A. Grateful acknowledgement is made in this connection of the generous facilities provided by Prof. G. W. Blaydes and Prof. J. N. Wolfe of the Ohio State University.

Abbreviations

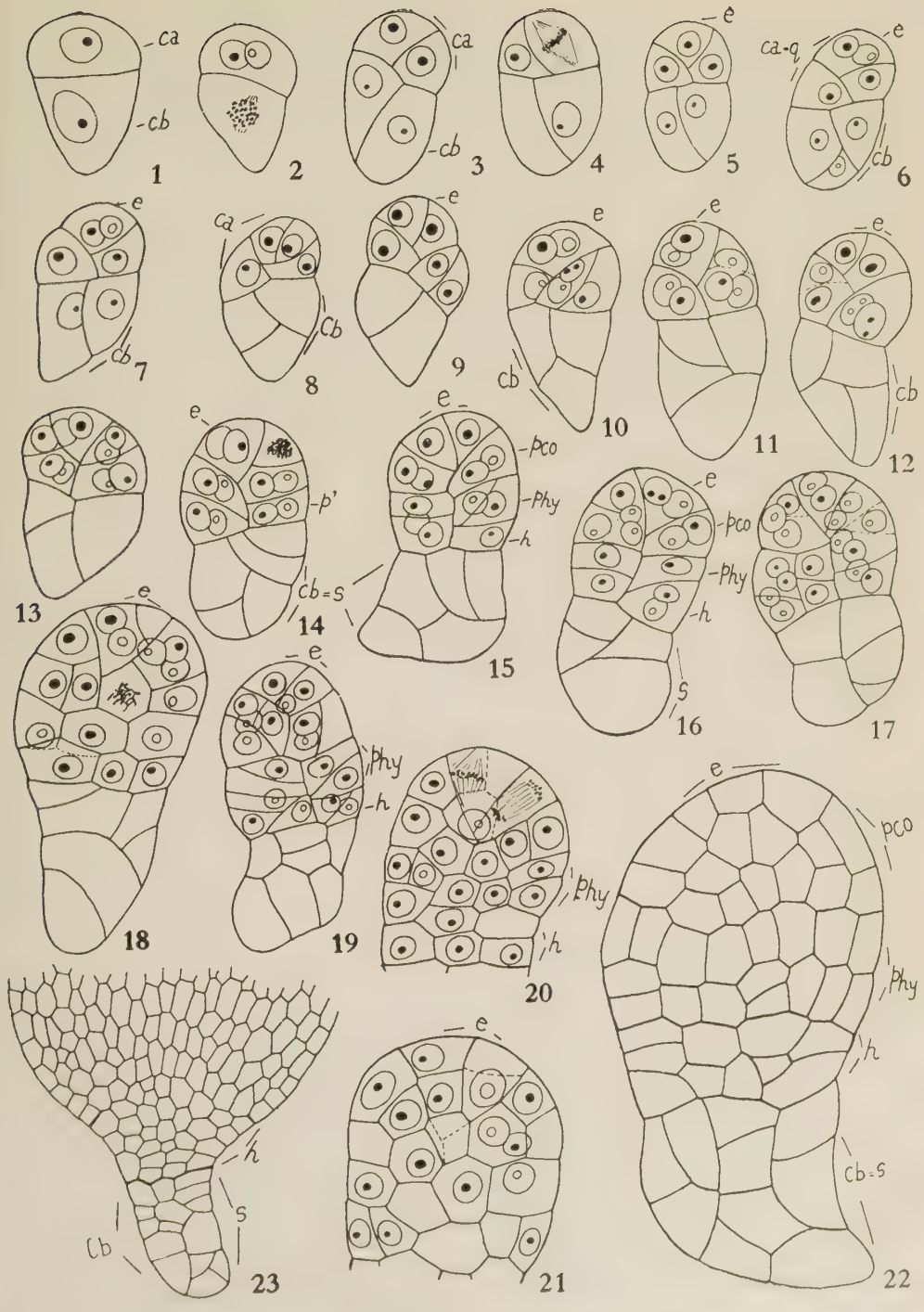
The following abbreviations originally employed by Souèges, the foremost authority on Plant Embryogeny, are now used widely in modern embryological researches and it has been suggested that they should be placed on an International basis. They are derived from original French expressions.

ca and *cb*, the apical and basal cells, being the daughter cells of fertilized egg.
cc and *cd*, the superior and inferior daughter cells of the apical cell.
ce and *cf*, the superior and inferior daughter cells of the cell, *cc*.
cg and *ch*, the superior and inferior daughter cells of the cell, *ce*.
ci, the basal cell of a tetrad.
m, the intermediate cell of a tetrad.
d and *f*, daughter cells of *m*.
n and *n'*, daughter cells of *ci*; *q*, quadrants; *l* and *l'* superior and inferior octants; *p'*, layer giving *phy* and *h*. *pco*, cotyledonary part; *pvt*, epicotyl or stem tip; *e*, epiphysis or epiphyseal tissue; *h*, hypophysis or hypophyseal tissue; *phy*, hypocotyledonary part; *icc*, initials of the cylinder of the root; *iec*, initials of the root epidermis; *co*, root cap; *s*, suspensor.

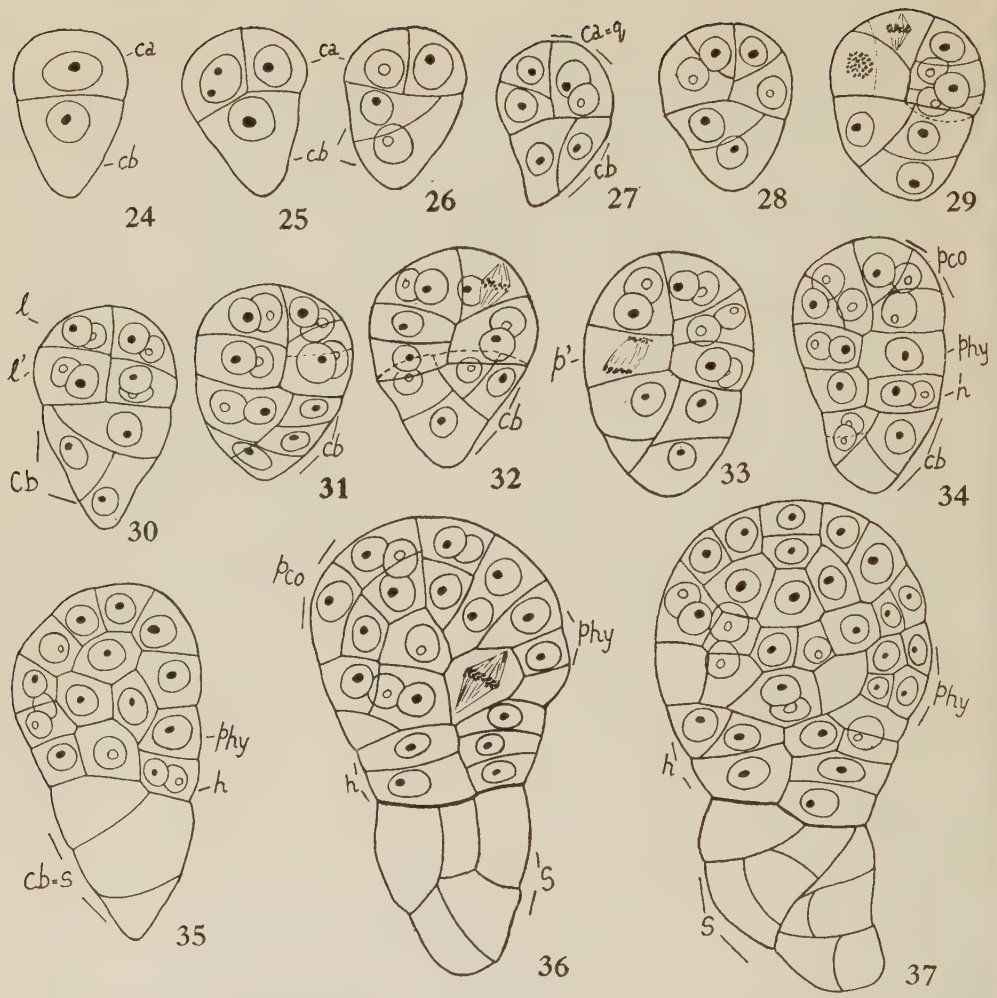
Observations

Cyamopsis psoralioides — The fertilized egg divides by a transverse wall to form two superposed cells *ca* and *cb* (Fig. 1). Oblique divisions in these two cells result in a tetrad of the category B_1 (Figs. 2, 3). Another oblique division in one of the derivative cells of *ca* separates the epiphysis initial (Figs. 4, 5). These early divisions conform to the fundamental type

*Paper presented to the Plant Embryology Section of the Eighth International Botanical Congress held in Paris during July 1954.



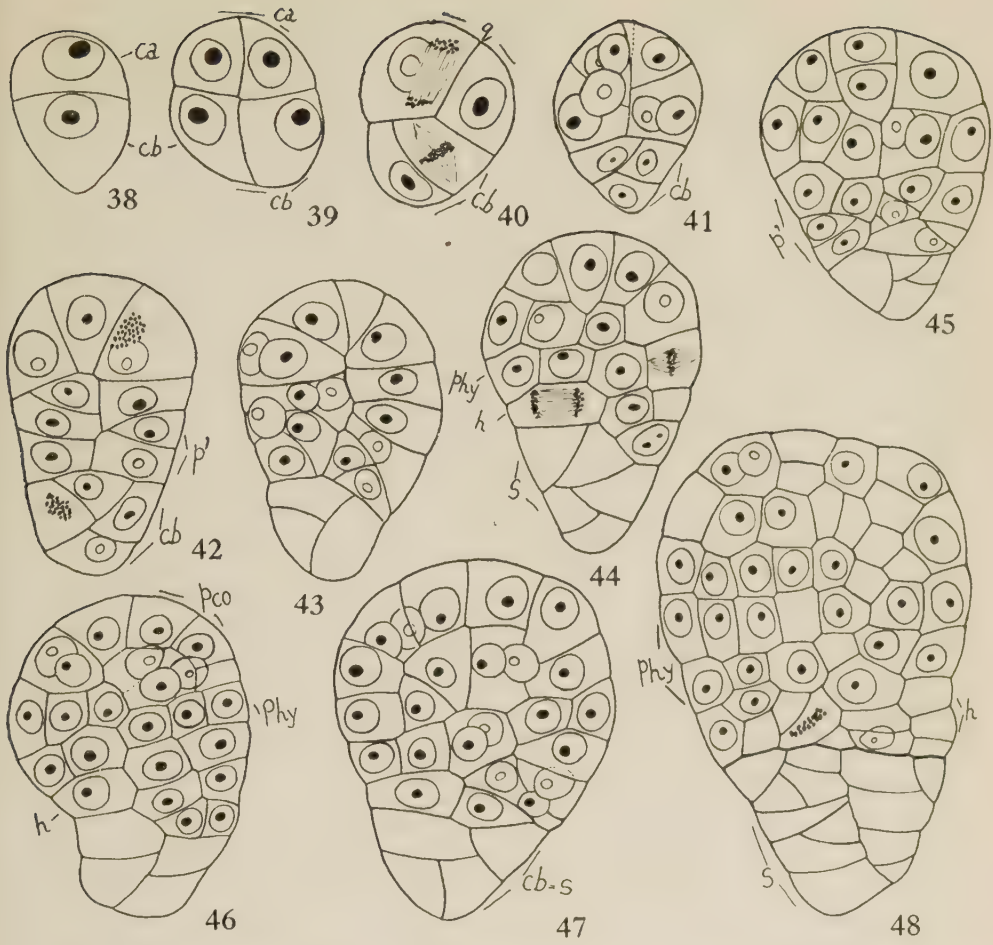
FIGS. 1-23. — Stages in development of embryo of *Cyamopsis psoralioides* (for abbreviations, see text). Figs. 1-22. × 600. Fig. 23. × 300.



FIGS. 24-37 — Stages in development of embryo of *Desmodium canadense* (for abbreviations, see text). All figs. × 600.

of *Trifolium* (Souèges, 1929). Subsequent divisions in the embryo are also oblique as represented in Figs. 6-12, but in the course of further development a layer *p'* becomes differentiated (Fig. 14) and from this layer are derived the hypocotyledonary and hypophyseal parts (Figs. 15, 16). The epiphyseal region is also evident at this stage but it may not always be clearly distinguishable from the cotyledonary part. The hypophyseal part eventually contributes to the extremity of the root including the root cap. Some of the important stages leading to the separation of these parts have been represented in Figs. 17-23. The suspensor is produced by the cell *cb* of the two-celled proembryo. The divisions in this cell do not keep pace with those in the apical cell with the result that the suspensor consists of a smaller number of cells. These cells are, however, larger in size and also do not stain deeply on account of paucity of cytoplasm.

In *Desmodium canadense* also the fertilized egg divides by a transverse wall (Fig. 24). A vertical division in each of the two cells *ca* and *cb* gives a tetrad of the A_1 category (Figs. 25, 26). The

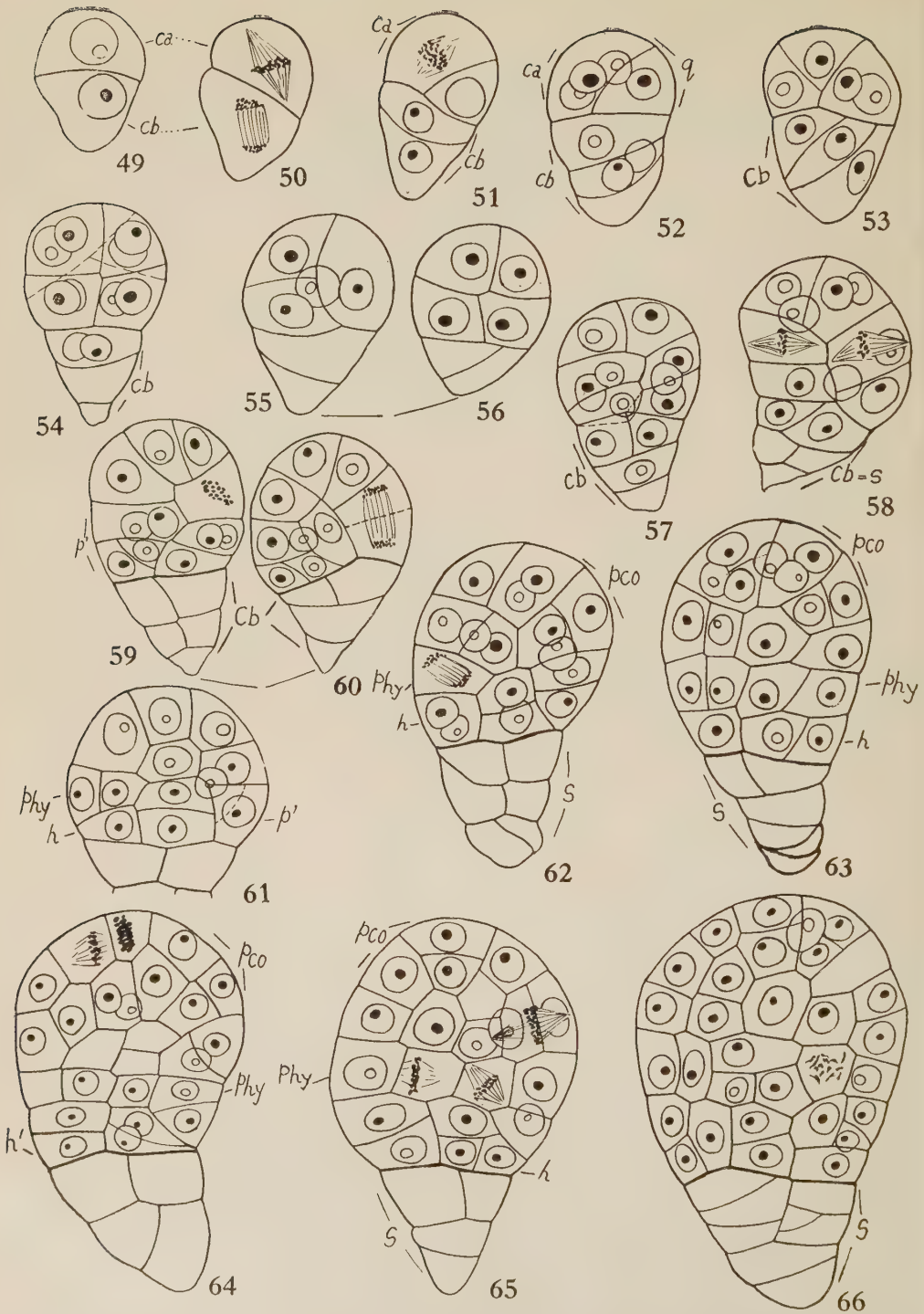


FIGS. 38-48 — Stages in development of embryo of *Desmodium canescens* (for abbreviations, see text). All figs. × 600.

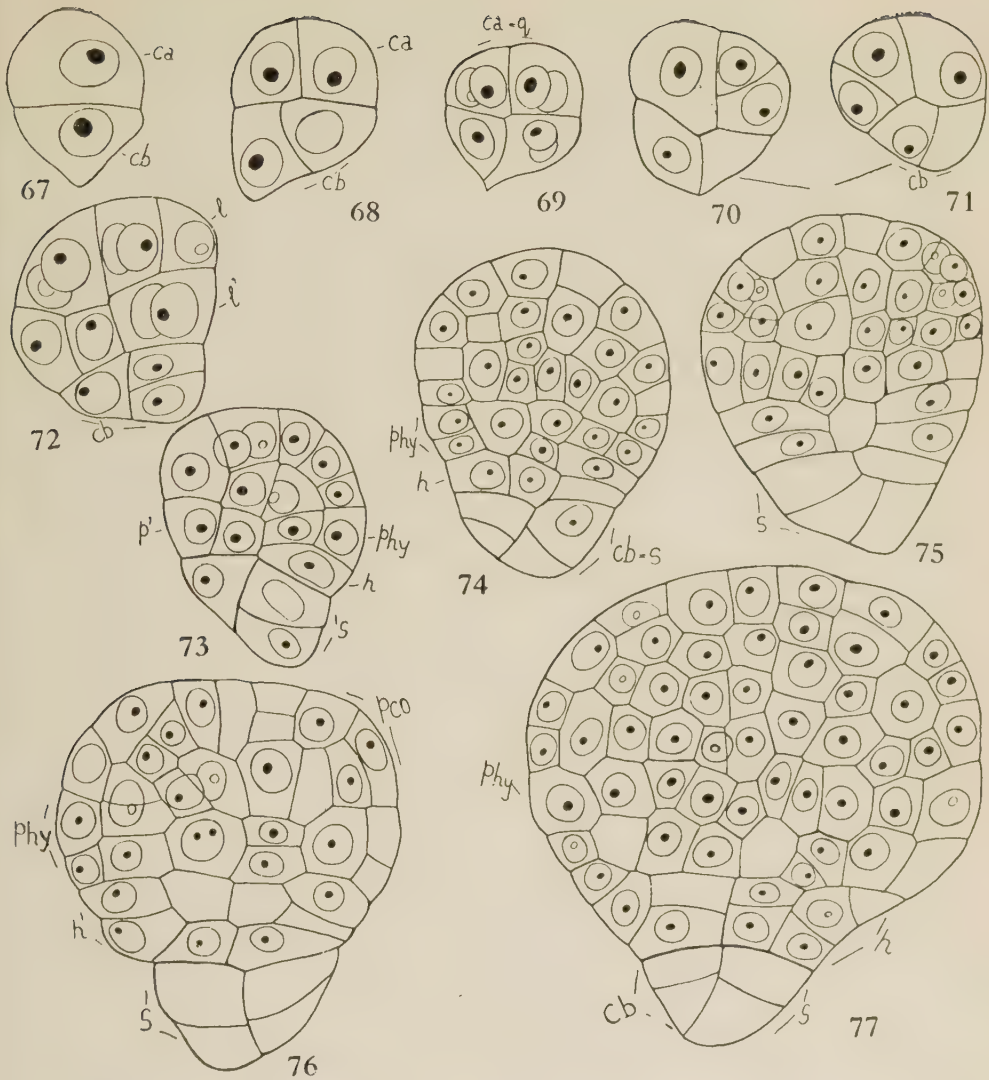
division in *cb* is usually oblique. The two juxtaposed cells derived from *ca* undergo another division to give rise to the quadrants. These four elements of the quadrants are not always disposed in the same manner as the plane of division leading to this stage varies (Figs. 27, 28). Similar variations have also been noticed in some members of the Hedysereae investigated by Souèges, viz. *Coronilla*, *Onobrychis* and *Ornithopus* (Souèges, 1947b, 1953a, b). The passage from the quadrants to the octants also shows some variations but usually the two tiers *l* and *l'* are clearly recognizable (Figs. 30, 31). Further divisions bring about the separation of the tiers *phy* and *h* which contribute to the hypocotyledonary and hypophyseal parts (Figs. 32-37). Meanwhile, *cb* divides to form a small group of vacuolated cells which constitute the suspensor. These observations are in conformity with those made by Souèges on other members of the Hedysereae.

Desmodium canescens is similar to *D. canadense* in its embryogeny and the important stages in development are illustrated in Figs. 38-48.

D. laevigatum shows some variations from the course of development described for the above two species. The fertilized egg undergoes a transverse division giving



FIGS. 49-66 — Stages in development of embryo of *Desmodium laevigatum*. Figs. 55-56 and 59-60 are adjacent sections of the same embryo (for abbreviations, see text). All figs. × 600.



FIGS. 67-77 — Stages in development of embryo of *Lespedeza violacea*. Figs. 70-71 are adjacent sections of the same embryo (for abbreviations, see text). All figs. × 600.

rise to *ca* and *cb* (Fig. 49). The division in the apical cell is oblique, whereas in the basal cell it may be transverse or oblique (Figs. 50, 51). The first tetrad may, therefore, be of the B_1 or B_2 category. The next division in the derivatives of *ca* results in the formation of quadrants (Fig. 52) but up to this time there is no differentiation of an epiphysis initial as is generally the case in members possessing a tetrad of the *B* type. Figs. 55 and 56 are drawn from adjacent sections of the same embryo and show the disposition of the octants, also represented in Fig. 54. Figs. 57 to 66 illustrate further stages in development which bring about the separation of the tiers *phy* and *h*, being the hypocotyledonary and hypophyseal parts respectively. As observed in other members, the divisions in the basal cell *cb* do not keep pace with those in the apical cell. As a result the suspensor, which is

derived solely from the basal cell, consists of only a small number of cells. *D. laevigatum* thus occupies an intermediate position between the first and the second embryogenic groups in the first period and may be considered to belong to an irregular type.

Lespedeza violacea, also belonging to the tribe Hedysereae, shows a more regular sequence of divisions in its embryogeny. The fertilized egg divides transversely forming the apical and the basal cells (Fig. 67). The next division in the apical cell is vertical but in the basal cell it is oblique (Fig. 68). The first tetrad is, therefore, of the A_1 category. The two juxtaposed daughter cells of the apical cell, by a vertical division, give rise to the quadrants (Fig. 69). Figs. 70 and 71 which represent adjacent sections of the same embryo illustrate the passage of the quadrants to the octant stage. The superior and the inferior octants are designated as *l* and *l'* in Fig. 72. Some later stages leading to the separation of the hypocotyledonary and hypophyseal parts are illustrated in Figs. 73-77. It is significant here that the suspensor, which is derived whole from the basal cell, remains short and consists of a very small number of cells.

From the description given above it is clear that all these members belong to the first period of the system of classification proposed by Souèges. *Cyamopsis* is to be referred to the second group and, since here the entire basal cell contributes to the suspensor, it must be assigned to megarchetype VI, along with the species of *Sesbania* (Galegeae) previously studied by the author (Anantaswamy Rau, 1951b). The fundamental embryonomic type, first recorded by Souèges for *Trifolium minus* (Souèges, 1929), has now been reported for several members of the tribes Genisteae, Loteae, Galegeae and Trifolieae, and as remarked by Souèges (1951a), from the embryogenic point of view, it is very difficult to delimit the above four tribes.

The tribe Hedysereae appears to be homogeneous in this regard, the members investigated so far conforming to the type originally recorded for *Coronilla minima* (Souèges, 1947 b), and belonging to the sixth megarchetype of the first embryogenic group in the first period. *Desmodium laevigatum* deviates slightly from the fundamental type and further investigations are necessary to evaluate the significance of this variation.

Discussion

The embryogenic system of classification proposed by Souèges, as is well known, comprises an indefinite number of periods designated the 'grand periods', the embryogenic groups and the megarchetypes. For a detailed account of this subject, reference must be made to the several publications of Souèges, particularly his essays on the embryogenic system (Souèges, 1939, 1948, 1951b). It is proposed to give here only a brief outline of this classification in so far as it relates to the position of the Papilionaceae. The fundamental basis for this classification is the nature of the first four cells of the proembryo, designated the tetrad, and the subsequent destiny of these cells in the organization of the embryo proper. Six categories of tetrads in three series are recognized by Souèges and these are as follows:

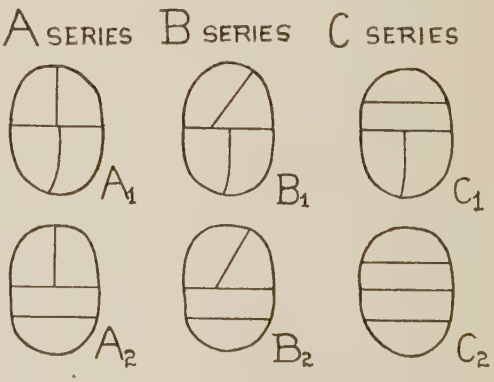


FIG. 78 — The six categories of tetrads.

The first four cells formed after two divisions in the fertilized egg constitute the first tetrad. When the apical cell of a two-celled proembryo undergoes a transverse division to give rise to two superposed cells *cc* and *cd*, the four cells derived in turn from *cc* and *cd* constitute the second tetrad, and this may again be one of the

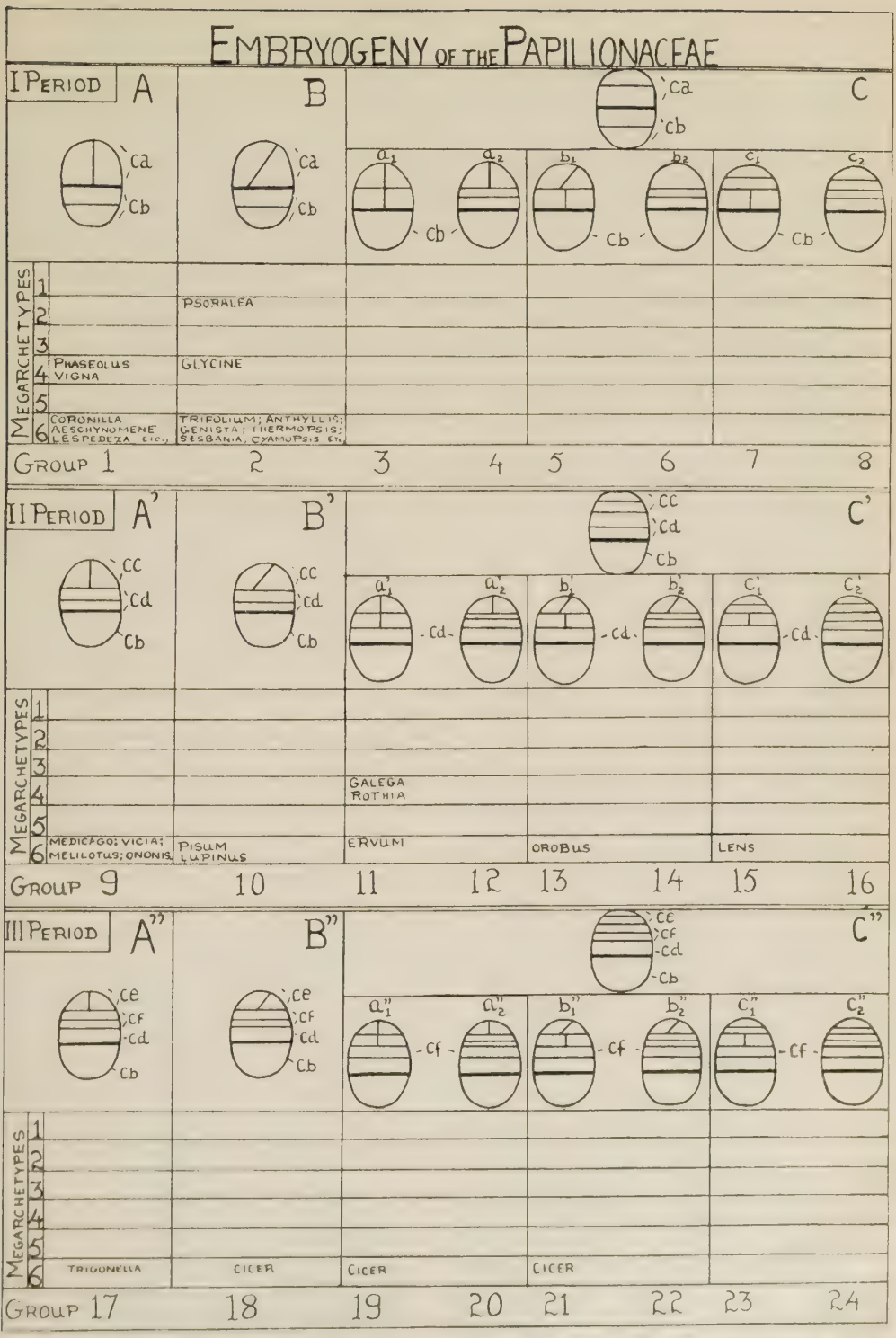


FIG. 79 — Chart to illustrate the principal results obtained in the embryogeny of the Papilionaceae.

six categories mentioned above. Similarly, the four cells resulting from the terminal cell *cc*, after it has undergone a transverse division to give *ce* and *cf*, form the third tetrad. These tetrads are significant in the recognition of the grand periods as can be seen in the following paragraphs.

The grand periods are designated first, second, third and so on, but at present representatives are known only for the first four periods. In each period there are eight groups and these are numbered serially from the first period onwards. Depending on the fate of the two superposed cells *ca* and *cb* in Period I, *cc* and *cd* in Period II, *ce* and *cf* in Period III, and so on, six megarchetypes are recognized. A schematic representation of this system of classification is given in Fig. 79.

In the first period the apical cell of the two-celled proembryo contributes to the embryo proper but the basal cell may also contribute in part to it or may give rise to the suspensor alone. In the second period the daughter cell *ca* of the two-celled proembryo undergoes a transverse division giving the two superposed cells *cc* and *cd*. Here the fate of the cell *cc* is similar to that of *ca* in the first period, likewise *cd* corresponds to *cb* and may contribute either partly or wholly to the upper part of the suspensor, the remaining part being derived from the basal cell *cb*. In the third period, the terminal cell *cc* divides transversely to form *ce* and *cf* which behave in the same way as *ca* and *cb* in the first period, and *cc* and *cd* in the second. In the third period, therefore, the suspensor is derived from the cells *cb* and *cd* and partly or wholly from *cf*. This scheme can be extended to the fourth and subsequent periods but, as stated earlier, only two members of the Fumariaceae are known to belong to the fourth period. Since the family Papilionaceae is not represented in this period, it is omitted from the accompanying chart.

The vertical columns in each period in the chart represent the embryogenic groups which are based on the form of the tetrads and their subsequent behaviour. In the *C* series of tetrads the sub-series are designated as a_1, a_2, b_1, b_2 and c_1, c_2 series.

The megarchetypes are recognized on the basis of the behaviour of the cells *ca* and *cb* in the first period, *cc* and *cd* in the second period, *ce* and *cf* in the third period and so on. In all these cases the upper of the two cells, namely *ca* or *cc* or *ce*, contributes wholly to the embryo proper while the other cell *cb* or *cd* or *cf*, may contribute partly to the embryo proper as shown below. The megarchetypes are accordingly numbered I to VI. It may be noted that the following table refers to the first period only. In the second and third periods *cd* and *cf* are substituted for *cb*. The abbreviations used are: *pvt*, stem tip; *phy*, the hypocotyledonary part; *icc*, the initials of the cylinder of the root; *iec*, the initials of epidermis of the root, *co*, the root cap and *s*, suspensor.

I. *cb* gives *pvt*+*phy*+*icc*+*iec*+*co*+*s*.
II. *cb* gives *phy*+*icc*+*iec*+*co*+*s*.
III. *cb* gives ½ *phy*+*icc*+*iec*+*co*+*s*.
IV. *cb* gives *iec*+*co*+*s*.
V. *cb* gives *co*+*s*.
VI. *cb* gives *s*.

The position of the Papilionaceae may now be discussed with reference to the scheme outlined above. Several members of the tribes, Trifolieae, Galegeae, Genisteae, Loteae, Hedysereae and Phaseoleae belong to the first period. Of these the Phaseoleae and the Hedysereae are represented in the first group, i.e. with a tetrad of the *A* category and the others in the second embryogenic group with a tetrad of the *B* category. The separation of an epiphysis initial at the quadrant stage is another unique feature of many members in the second group. Further, it may be noted that most of the species coming under these groups belong to the sixth megarchetype which appears to be characteristic of certain tribes, like the Hedysereae and the Genisteae. The Phaseoleae are in the fourth megarchetype with *Phaseolus* and *Vigna* appearing in the first group and *Glycine* in the second.

This may be summarized thus:

First Period

Series A (Group 1) — Megarchetype IV: *Phaseolus*, *Vigna* (Phaseoleae). Megarchetype VI: *Coronilla*, *Aeschynomene*; *Onobrychis*, *Ornithopus*, *Desmodium* spp., *Lespedeza* (Hedysereae).

SERIES B (Group 2)— Megarchetype II: *Psoralea* (Galegeae). Megarchetype IV: *Glycine soja* (Phaseoleae). Megarchetype VI: *Trifolium* (Trifolieae); *Dorycnium, Tetragonolobus, Anthyllis* (Loteae); *Genista, Ulex, Sarothamnus* (Genisteae); *Astragalus, Colutea, Sesbania* spp., *Cyamopsis* (Galegeae); *Thermopsis* (Podalyrieae).

In the second period appear several members of the tribes Trifolieae and Vicieae. *Lupinus* and *Rothia*, of the Genisteae, and *Galega* of the Galegeae also belong here. *Medicago, Ononis* and *Melilotus* of the Trifolieae belong to the sixth megarchetype in the ninth group. An analysis of the embryogeny of these members illustrates clearly the superposition of this type over the first group represented by *Coronilla* in the same megarchetype. The principle of correspondence of forms relating to this is discussed later. *Pisum* and *Lupinus* appear in the tenth group and megarchetype VI. In the eleventh group the fourth megarchetype is represented by *Galega* and *Rothia*. Two other members of the tribe Vicieae, *Orobus* and *Lens*, belong to the sixth megarchetype in the thirteenth and fifteenth groups respectively. The important results obtained in this group are summarized here:

SECOND PERIOD

SERIES A (Group 9)— Megarchetype VI: *Medicago*; *Melilotus*; *Ononis* (Trifolieae); *Vicia* spp. (Vicieae).

SERIES B (Group 10)— Megarchetype VI: *Pisum* (Vicieae); *Lupinus* (Genisteae).

SERIES C (Group 11)— Megarchetype IV: *Galega* (Galegeae); *Rothia* (Genisteae). Megarchetype VI: *Ervum* (Vicieae). (Group 13)— Megarchetype VI: *Orobus* (Vicieae). (Group 15)— Megarchetype VI: *Lens* (Vicieae).

Thus only two tribes of the Papilionaceae are predominantly represented in the second period. Further, most of the members appear in the megarchetype VI. A similar feature is seen in the first period also where certain tribes dominate in certain groups and in the sixth megarchetype.

Only two genera of the family, viz. *Trigonella* (Trifolieae) and *Cicer* (Vicieae) are at present known to belong to the third period. *Trigonella foenum-graecum* (Anantaswamy Rau, 1950) and *T. caerulea* (Crété, 1953) appear in the seventeenth group and megarchetype VI, in the same corresponding position as *Melilotus* and *Medicago* in the second period. It is significant that all these genera belong to the same tribe, Trifolieae. *Cicer*, belonging to the Vicieae, comes under the eighteenth group corresponding to the position occupied by *Pisum* in the second period, although certain variants of *Cicer* also appear in the nineteenth and twenty-first groups. Here again it is significant that other genera of the same tribe occupy corresponding positions in the second period.

In conclusion it may be stated that a careful and detailed investigation of the embryogeny of a large number of representative species of the Papilionaceae is necessary for the elucidation and evaluation of the systematic relationships of the tribes and genera of this important family. An analysis of the embryonomic data would serve to indicate the significance of the principle of correspondence of forms enunciated by Souèges (1947). The following account illustrates this point as well as the superposition of certain types referred to earlier. The principle of the correspondence of forms takes into account the sequence of divisions in the early stages of the proembryo. In the first cell generation, two superposed cells are met with; in the second a group of four cells which may be of any one of the six types of tetrads; in the third generation, the quadrants or the homologous elements produced by the two superior cells of the tetrad; in the fourth generation the octants or the elements derived by division of the quadrants. It is obvious that the first generation referred to in this principle consists of the two cells derived from the fertilized egg in the first period; from the apical cell in the second period and from the terminal cell *cc* in the third period. The following is an analysis of the embryonomic data obtained from the three representatives of the three periods, viz. *Coronilla*; *Medicago* and *Trigonella*.

FIRST PERIOD: *Coronilla minima* (Souèges, 1947c)

First generation

Proembryo of two cells disposed in two tiers.
- *ca* gives *pco*+*pvt*+*phy*+*icc*+*iec*+*co*.
- *cb* gives *s*.

Second generation (*tetrad*)

Proembryo of four cells disposed in two tiers.
- destiny of *ca* and *cb* same as above.

Third generation (*quadrants*)

Proembryo of eight cells disposed still in two tiers.
- *ca* (*q*): destiny same as above.
- *cb*: same as above.

Fourth generation (*octants*)

Proembryo of about sixteen cells disposed in three tiers.
- *l* (superior octants) gives *pco*+*pvt*.
- *l'* (inferior octants) gives *phy*+*icc*+*iec*+*co*.
- *cb* gives *s*.

SECOND PERIOD: *Medicago lupulina* (Souèges, 1929)
(This analysis does not include the cell *cb*)

First generation

Proembryo of two cells disposed in two tiers.
- *cc* gives *pco*+*pvt*+*phy*+*icc*+*iec*+*co*.
- *cd* gives *s* (upper part, lower part from *cb*).

Second generation (*second tetrad*)

Proembryo of four cells disposed in three tiers.
- *cc* gives *pco*+*pvt*+*phy*+*icc*+*iec*+*co*.
- *m*+*ci* give *s* (upper part).

Third generation (*quadrants*)

Proembryo of eight cells disposed in four tiers.
- *cc* gives *pco*+*pvt*+*phy*+*icc*+*iec*+*co*.
- *m*+*n*+*n'* give *s* (upper part).

Fourth generation (*octants*)

Proembryo of about fourteen cells disposed in five tiers.
- *l* gives *pco*+*pvt*.
- *l'* gives *phy*+*icc*+*iec*+*co*.
- *m*+*n*+*n'* give *s* (upper part).

THIRD PERIOD: *Trigonella foenum-graecum* (Anantaswamy Rau, 1950)
(This analysis does not include the cells *cb* and *cd* which give *s*)

First generation

Proembryo of two cells disposed in two tiers.
- *ce* gives *pco*+*pvt*+*phy*+*icc*+*iec*+*co*.
- *cf* gives *s* (upper part).

Second generation (*third tetrad*)

Proembryo of four cells disposed in three tiers.
- *ce* gives *pco*+*pvt*+*phy*+*icc*+*iec*+*co*.
- *d*+*f* give *s* (upper part).

Third generation (*quadrants*)

Proembryo of eight cells disposed in three tiers.
- *ce* (*q*): same as above.
- *d*+*f*: same as above.

Fourth generation (*octants*)

Proembryo of about sixteen cells disposed in four tiers.
- *l* gives *pco*+*pvt*.
- *l'* gives *phy*+*icc*+*iec*+*co*.
- *d*+*f* give *s* (upper part),

From the above analysis, it is clear that the embryonomy of these representatives of the three grand periods is identical (variation in the nature of the tetrad in A series is possible, i.e., it may be of the A_1 or A_2 category). The potentialities of the cells *ca* and *cb* in the first period are shifted to the cells *cc* and *cd* in the second and to *ce* and *cf* in the third period. A similar correlation of data is possible in the B series also where *Trifolium* in the first period (second group), *Pisum* in the second period (tenth group) and *Cicer* in the third period (eighteenth group) significantly illustrate the principle of correspondence of forms.

As has been pointed out earlier, investigation of a large number of members of a family employing the rigorous methods of embryogenic study would contribute considerably to our understanding of the relationships of the genera, tribes and families. With particular reference to the Papilionaceae, the following statement of Johansen (1950) may be quoted here: "The embryonomy of species belonging to the Papilionaceae is perplexing; a great deal of careful study of all the genera is required before the situation can be fully elucidated."

Summary

The embryogeny of five members of the Papilionaceae, *Cyamopsis psoralioides* (Galegeae), *Desmodium canadense, D. canescens, D. laevigatum* and *Lespedeza violacea* (Hedysereae) has been studied. It is found that all these species are ranged in the first period of the embryogenic system of classification. *Cyamopsis*, by virtue of its first tetrad of a B category, is placed in the second group and megarchetype VI and the rest of the members in the first group and in the same megarchetype.

A brief discussion of the importance of the Papilionaceae in the system of classification and an analysis of embryonomic data to illustrate the principle of correspondence of forms are also included in the paper.

The studies reported here were carried on in the Botany Department of the Ohio State University, Columbus, Ohio, U.S.A., through the generous award of the Mrs. Mary S. Muellhaupt post-doctoral scholarship for 1953-54 by the Graduate School of the Ohio State University. Sincere gratitude is expressed to Professors B. S. Meyer and G. W. Blaydes for the extraordinary generosity and consideration they extended to the author during his stay in Columbus. Prof. E. C. R. Souèges of the University of Paris and Prof. P. Maheshwari of the University of Delhi have given continuous help and encouragement in these studies and recently the author had the benefit of personal discussions with them in Paris. He is deeply indebted to them. Finally, gratitude is expressed to the authorities of the University of Mysore for placing him on deputation for this study abroad.

Literature Cited

Anantaswamy Rau, M. 1950. Development of the embryo in *Trigonella foenum-graecum* L. J. Indian Bot. Soc. **29**: 210-213.

— 1951a. Development of the embryo in *Aeschynomene indica* Linn. New Phytol. **50**: 124-126.

— 1951b. Development of the embryo in some members of the Papilionaceae. Phytomorphology **1**: 80-86.

Crété, P. 1953. Embryogénie des Papilionacées. Développement de l'embryon chez le *Trigonella caerulea* Ser. C.R. Acad. Sci. Paris **236**: 740-742.

Johansen, D. A. 1950. "Plant Embryology." Chronica Botanica Co., Waltham, Mass., U.S.A.

Souèges, R. 1929. Recherches sur l'embryogénie des Légumineuses. Bull. Soc. bot. France **76**: 338-346.

— 1939. Embryogénie et classification. 2e Fascicule., Paris.

— 1947a. Embryogénie végétale.— Le principe de la correspondence des formes. C.R. Acad. Sci. Paris **224**: 312-314.

— 1947b. Embryogénie des Papilionacées. Développement de l'embryon chez le *Coro-*

nilla minima L. C.R. Acad. Sci. Paris **224**: 1254-1256.
— 1947c. Les lois du développement de l'embryon chez le *Coronilla minima* L. Bull. Soc. bot. France **94**: 272-276.
— 1948. Embryogénie et classification. 3e fascicule. Paris.
— 1951a. Embryogénie des Papilionacées. L'embryon et le suspenseur chez l'*Anthyllis vulneraria* L. C.R. Acad. Sci. Paris **232**: 581-584.
— 1951b. Embryogénie et classification. 4e fascicule. Paris.
— 1953a. Embryogénie des Papilionacées. Développement de l'embryon chez l'*Onobrychis sativa* Lam. C.R. Acad. Sci. Paris **237**: 450-452.
— 1953b. Embryogénie des Papilionacées. Développement de l'embryon chez l'*Ornithopus perpusillus* L. C.R. Acad. Sci. Paris **237**: 1199-1201.
— & Crété, P. 1952. Les acquisitions les plus récentes de l'embryogénie des Angiospermes. Ann. Biol. Paris **28**: 9-45.

THE GAMETOPHYTE AND YOUNG SPOROPHYTE OF *BOTRYCHIUM JAPONICUM* UND

Y. NOZU
Botanical Institute, University of Tokyo, Japan

Studies on the gametophytes of several species of *Botrychium* have been carried out by Hofmeister (1854), Milde (1858), Jeffrey (1898), Lyon (1905), Bruchmann (1906), Campbell (1921, 1922) and others. These were summarized by Campbell (1911) and Bower (1926). Gametophytes of species of the "ternate" group, however, have not been fully investigated by these authors. In 1905 Lyon studied the embryo of *Botrychium obliquum* in which she found a suspensor but no pronounced lateral cotyledon. She, therefore, proposed to separate it as the type of a new genus, *Sceptridium*. Campbell (1921) published a detailed account of the gametophyte and embryo of *B. obliquum*, supplementing the observations made by Lyon. He reported that it resembled *B. virginianum* in possessing a well developed cotyledon but differed in the endogenous origin of the root, the bipolar arrangement of the cotyledon, and the presence of a suspensor.

In the autumn of 1951 and 1953 the writer fortunately discovered several prothalli of *Botrychium japonicum* at two localities near Tokyo. Most of the prothalli bore young sporophytes. Since some marked differences were noted between this species and the ones previously investigated, a brief account is given below.

Gametophyte

The gametophyte of *Botrychium japonicum* is subterranean and grows at a depth of 3-7 cm. below the soil level. It is relatively slender and bears a close resemblance to an insect worm. The gametophyte is 5-7 mm. long and half as wide, and is smaller in size than that of *B. virginianum*. It is dorsiventral with the young sporophyte situated on the dorsal surface. The ventral surface bears numerous, irregularly arranged long rhizoids. The hyphae of an endophytic fungus occupy a large part of the inner tissue on the ventral side of the gametophyte. They are absent from the dorsal meristematic region (Fig. 4, C, D). In this respect *B. japonicum* closely resembles *B. virginianum* and *B. obliquum*.

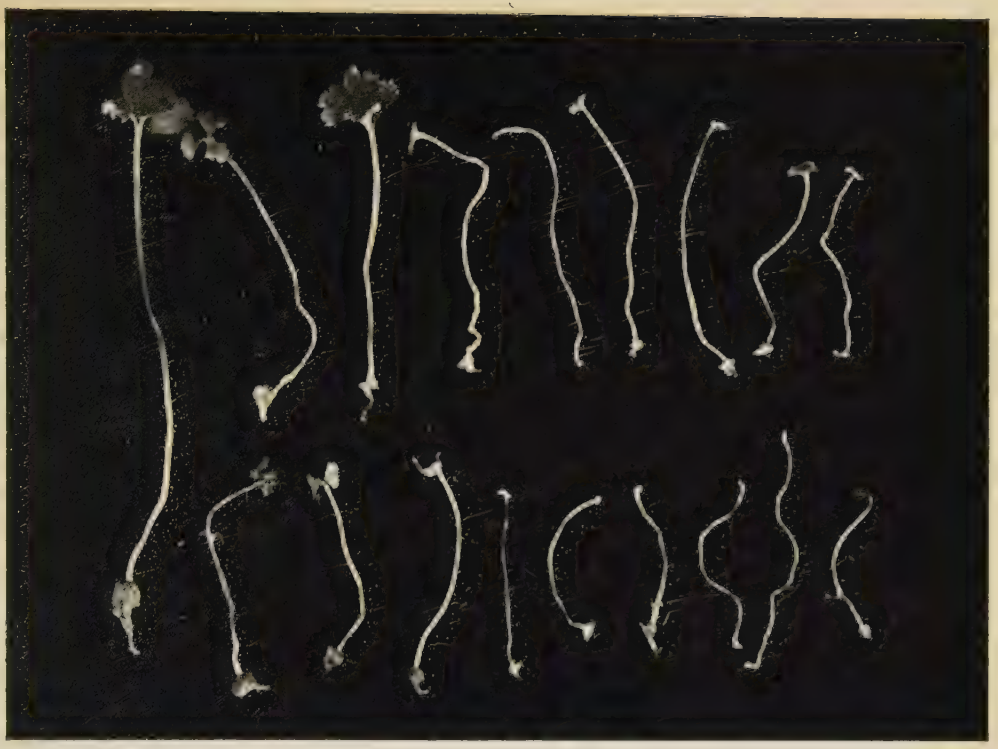


FIG. 1 — Young sporophytes of *Botrychium japonicum* attached to the gametophyte. ×1·5.

The gametophyte is monoecious and protandrous. The antheridia are borne only upon the dorsal median ridge and their development seems to be similar to that in other species (Fig. 4, G). They are capped by two layers of cover cells. Frequently empty antheridia with degenerated opercular cells were seen. Unfortunately the material was not favourable for a study of spermatogenesis and living spermatozoids could not be investigated.

The archegonia appear on the flanks of the antheridial ridge (Fig. 4, C). The mature archegonium is much like that of other species. The presence of a ventral canal cell in the archegonium is noteworthy, specially because it has not so far been demonstrated in *Botrychium*. It seems probable that the ventral canal cell is cut off just before the archegonium opens. In this respect the archegonia show some resemblance to *B. obliquum*.

Embryo

The development of the embryo could not be traced fully. Some divisions seem to occur in the suspensor before the embryonal cell divides (Fig. 4, F). The suspensor soon increases in size and becomes multicellular, resulting in an irregular and oblique structure. Subsequently the cotyledon and stem apex also become recognizable. The leaf arises as a group of meristematic cells close to the stem apex. The tissue below the stem apex is usually composed of a group of meristematic cells.

Young Sporophyte

The first leaf emerges from the upper side of the gametophyte and is soon differentiated into a petiole and a lamina. A young sporophyte, still attached to

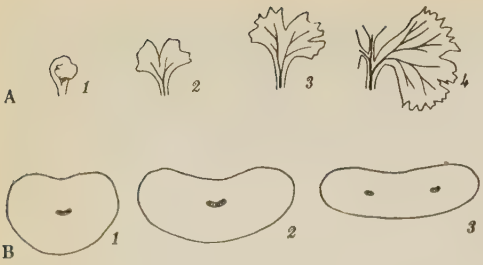


FIG. 2 — *A*, juvenile leaves showing successive steps in progression of dichotomous venation. *B*, t.s. petiolar base (1), upper part of petiole (2), and base of lamina (3).

the prothallium, is provided with a long, narrow, soft and colourless petiole which looks much like a piece of white tape (Fig. 1).

The leaf tip is simple and unbranched when young (Fig. 2, A-1). It soon acquires the shape of a *Ginkgo* leaf with even margins (Fig. 2, A-2). The venation is not visible at this stage as the leaf is relatively thick. In earlier stages chloroplasts are present in small numbers. At a somewhat later stage the juvenile leaves show a scorpioid sympodium type of venation, as reported by Bower (1926) in the young leaves of *Osmunda* (Fig. 2, A-3). The venation of successive juvenile leaves changes from dichotomous to ternate passing through sympodial branching (Figs. 1, 2, A). As the first leaf

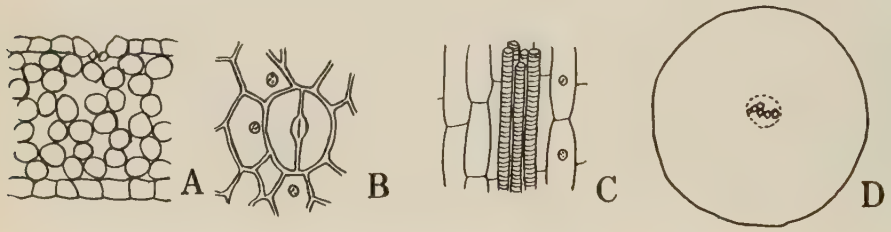


FIG. 3 — *A*, part of t.s. immature lamina; *B*, stoma, surface view; *C*, l.s. primary root, showing spiral tracheids; *D*, t.s. primary root showing a diarch stele. ×150.

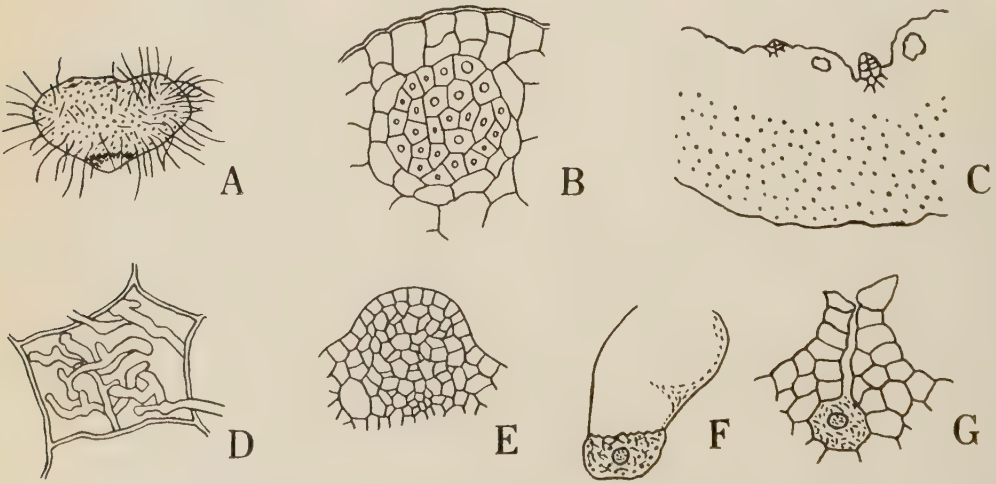


FIG. 4 — *A*, mature gametophyte with numerous rhizoids. ×6; *B*, median section of an antheridium; *C*, part of l.s. old gametophyte showing an endophytic fungus on ventral side and two archegonia on dorsal side; *D*, a cell from the gametophyte showing the endophytic fungus; *E*, l.s. stem apex showing meristematic cells; *F*, unicellular embryo just before the first division; *G*, archegonium with open neck.

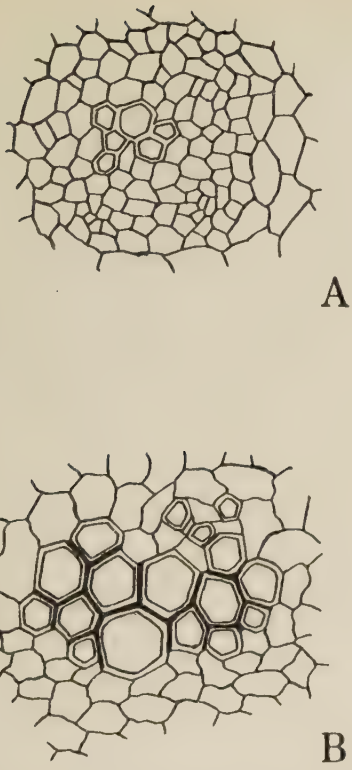


FIG. 5 — *A*, t.s. stele of primary root. × 320; *B*, t.s. stele of first leaf base. ×320.

matures, the petiolar trace becomes continuous with that of the root.

The base of the petiole, which is more or less flattened at the cotyledon stage, later becomes comparatively cylindrical. The vascular bundle divides at the base of the lamina into two strands (Fig. 2, B-1, 2, 3). Each of these may further divide in the median region of the lamina, so that several bundles are sometimes seen in a cross-section through this part. Venation of this type is rarely met with in species belonging to the section *Euophioglossum* of the genus *Ophioglossum*.

The structure of the young lamina is exceedingly simple. The two epidermal layers consist of thin-walled, more or less flattened cells (Fig. 3, A). Stomata develop on both the surfaces (Fig. 3, A, B). The mesophyll consists of thin-walled chlorenchyma cells with inter-cellular spaces (Fig. 3, A).

The earlier stages in the development of the primary root were not seen. In advanced stages the root is provided with a conspicuous apical cell, resembling that in other species of Ophioglossaceae. A root cap is present, but at first it is indistinguishable from the other tissues of the root. The primary vascular bundle of the root develops.

In later stages, when the young leaf has attained a fair size, the primary root begins to grow rapidly and penetrates through the gametophyte, soon emerging from its ventral surface (Fig. 4, A). In *B. obliquum* the primary root develops later than in *B. japonicum*. The anatomical structure is rather simple. Beneath the epidermis is the cortex consisting of 3-5 layers of parenchymatous calls. There is a diarch stele comprising several elements of xylem and phloem.

Summary and Conclusion

1. The gametophyte and young sporophyte of *Botrychium japonicum*, which belongs to the " ternate " group, resemble those of *B. obliquum* in various external features, organization of sex organs, presence of a suspensor, cotyledon with ternate lamina, bipolar endogenously originating root and leaf and endogenous origin of the root.

2. *Botrychium japonicum* differs from *B. obliquum* in the size of the gametophyte, certain features of the rhizoids, the vascular supply of the petiole, the development of the root and in the projected portion of the archegonia.

3. The vascular supply of the young sporophyte differs in the two species. This seems to be due to a difference in the degree of the dependence on the symbiotic fungus.

4. The vascular supply of the young sporophyte is composed exclusively of leaf and root traces. The part of the stele, where the strands run close together, was designated as " Phyllomophore " by the writer (1950).

5. Although some workers accept Lyon's view, this cannot be considered free from doubt since so far a suspensor has been known only in a few species of *Botrychium*.

Literature Cited

BITTER, G. 1902. Ophioglossales. In Engler & Prantl's "Die natürlichen Pflanzenfamilien". Leipzig.

BOWER, F. O. 1926. "The ferns (Filicales)." Vol. 2. Cambridge.

BRUCHMANN, H. 1906. Über das Prothallium und die Sporenpflanze von *Botrychium lunaria* Sw. Flora **96**: 203: 227.

CAMPBELL, O. H. 1911. The Eusporangiatae. Carnegie Inst., Washington, Publ. 140.

— 1921. Gametophyte and embryo of *Botrychium obliquum* Mühl. Ann. Bot. **35**: 141-158.

— 1922. The gametophyte and embryo of *Botrychium simplex* Hitch. Ann. Bot. **36**: 441-455.

CLAUSEN, R. T. 1937. "A Monograph of the Ophioglossaceae."

COPELAND, E. B. 1947. "Genera Filicum." Chronica Botanica Co., Waltham, Mass., U.S.A.

EAMES, A. J. 1936. "Morphology of Lower Vascular Plants." New York.

HOFMEISTER, W. 1854. Keimung des *Botrychium lunaria* Sw. Bonplandia **3**: 331-335.

JEFFREY, E. C. 1898. The gametophyte of *Botrychium virginianum*. Trans. Canad. Inst. **5**: 1-32.

LYON, H. L. 1905. A new genus of Ophioglossaceae. Bot. Gaz. **40**: 455-458.

MILDE, J. 1858. Über *Botrychium lanceolatum* Angstr. Bot. Z. **16**: 69-70.

VASCULAR ANATOMY OF THE FLOWER OF CERTAIN SPECIES OF THE AMARANTACEAE WITH A DISCUSSION OF THE NATURE OF INFLORESCENCE IN THE FAMILY

T. S. BAKSHI* & S. L. CHHAJLANI

Department of Botany, University of Delhi & Department of Botany, Birla College, Pilani

Introduction

In 1934 Joshi & Rao published a more or less detailed account of the floral anatomy of *Digera arvensis* Forsk. Their conclusions regarding the morphology and early history of the family Amarantaceae have, however, been shown to be unsatisfactory in some respects (Bakshi, 1952). A comparative study of some members of the family, including a re-investigation of *D. arvensis*, therefore, seems to be called for.

The present study deals with four species: *D. arvensis*, *Pupalia lappacea* Moq., *Achyranthes aspera* Linn. and *Gomphrena globosa* Linn. the material of which was collected at Pilani during July-August 1952, and was preserved in formalin-acetic-alcohol. A few spikes of *Achyranthes aspera* were collected by one of us (T.S.B.) from the Delhi University campus in June 1952. The flowers were passed through the usual dehydration and infiltration series. Sections were cut 8-10 μ thick and stained in safranin and fast green as well as in crystal violet and erythrosin. The latter combination gave excellent results.

Observations

Digera arvensis

EXTERNAL MORPHOLOGY — The subsessile flowers of *D. arvensis* are arranged in spikes about 1-4 in. long. Each flower is subtended by a bract and two bracteoles which are smaller than the perianth segments. Each bracteole has in its axil a small crested structure inappropriately called a 'scale'. To the naked eye the so-called 'scale' appears as a dichotomously branched green structure which

*Now at Department of Botany, State College of Washington, Pullman, Washington, U.S.A.

together with its counterpart of the other side encloses the fruit at maturity.

The perianth consists of five segments, the two outer, which alternate with the bracteoles, are larger than the three inner. The outer ones are reported to have 5-9 (Hooker, 1885) or more than 9 (Joshi & Rao, 1934) nerves while the inner are said to be 2-4 (Hooker, 1885) or only 1-nerved (Joshi & Rao, 1934). In our material the outer showed 5-10 nerves and the inner 1-3. There are five stamens which, according to Hooker, are free, but microtome sections reveal the presence of a short staminal tube at the base. The ovary is oblong and truncate, the style filiform, and the stigma bilobed and recurved. There is a solitary sub-basal ovule in each ovary.

Vascular Anatomy — At the time of emergence of the first bracteole trace the vascular tissue in the pedicel shows an almost complete ring of phloem with xylem broken up into three distinct groups (Fig. 2). This is soon followed by another trace that enters the 'scale' arising in the axil of this bracteole (Fig. 3). The trace for the second bracteole is given out on the opposite side. As this passes out, it leaves behind some vascular tissue for the second 'scale' (Figs. 3-8). Each 'scale' trace continues undivided up to the level of the appearance of the carpellary traces. Here it gives out two branches, one after the other, and then fades away (Figs. 11-13). Each branch behaves like the parent 'scale' trace and gives the 'scale' the appearance of a dichasium (Fig. 1).

While the trace for the second bracteole is still in the axial cortex the two outer perianth segments begin receiving their vascular supply. Each segment gets three traces emerging independently from a common gap (Fig. 4). As they diverge out they divide and redivide forming 6-10 vascular bundles in the segment (Figs. 4-11). The inner three perianth segments receive one trace each in order of their emergence and this shows gradual reduction in its branching. In the third segment, for instance, the vascular bundle after traversing some distance, gives off a small lateral branch on the upper side which bifurcates higher up (Figs. 10-14). In p_4 the vascular bundle gives out a lateral branch which, however, remains undivided. In p_5 the single bundle never divides (Fig. 14).

The traces to the stamens originate in the order a_1-a_5, the trace to appear first being situated opposite to p_1, the second opposite to p_2, and so on. Each staminal trace remains *unbranched* throughout its course (Figs. 8-14).

The remaining stelar tissue gives off two dorsal bundles at slightly different levels (Fig. 10). They enter the ovary wall and continue right up to the stigma. The rest of the stelar tissue continues as a ventral bundle which enters the funiculus and fades away at the base of the only ovule.

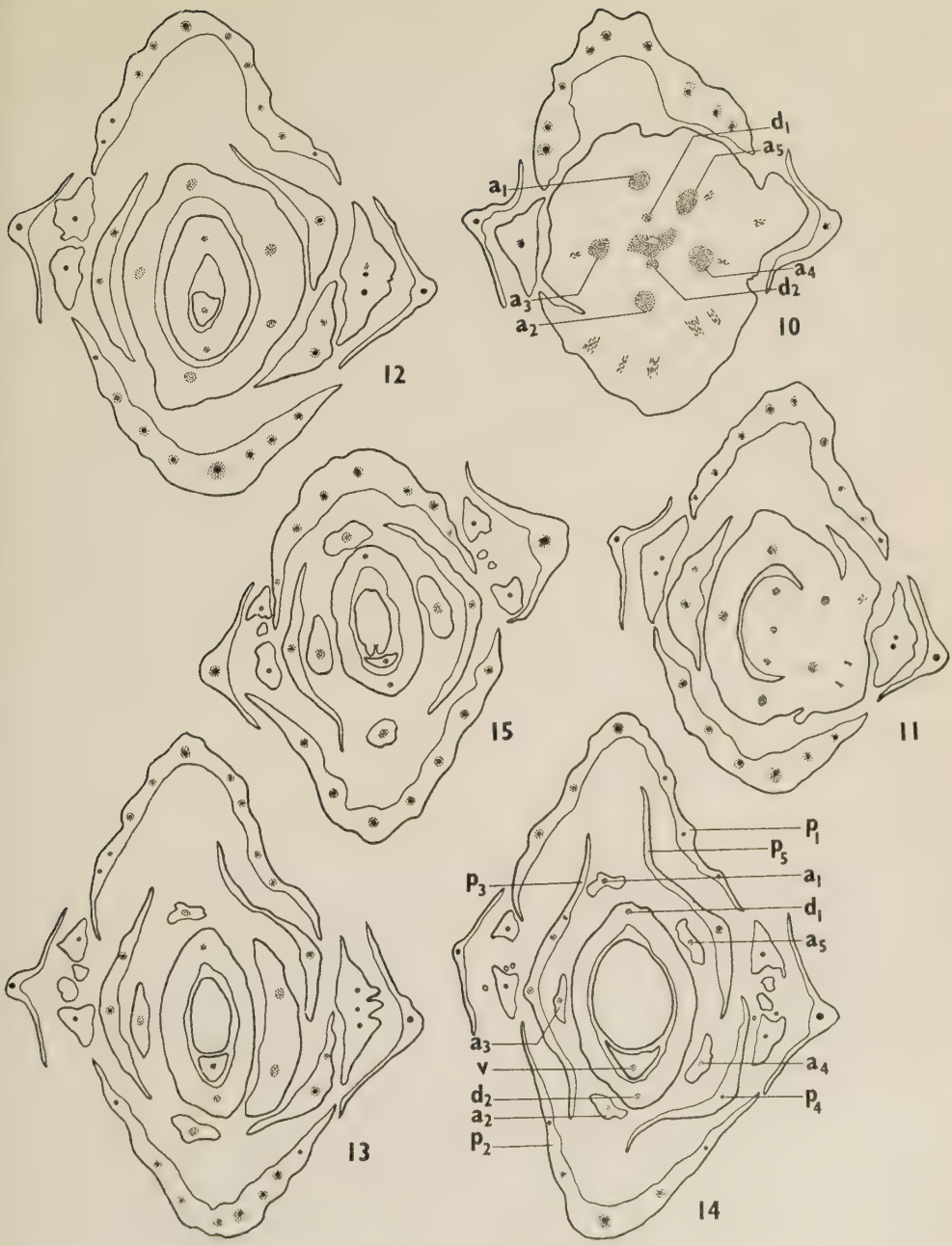
Pupalia lappacea

External Morphology — The flowers of *P. lappacea* occur in spicate clusters arranged into a spike. Each cluster consists of a central fertile and two to six sterile flowers. There are five perianth segments, two outer and three inner. They enclose five stamens whose filaments are united into a very short staminal tube at the base. There is a unilocular ovoid ovary with a slender style, and a slightly bilobed stigma. The ovule is solitary and pendulous from a long sub-basal funicle.

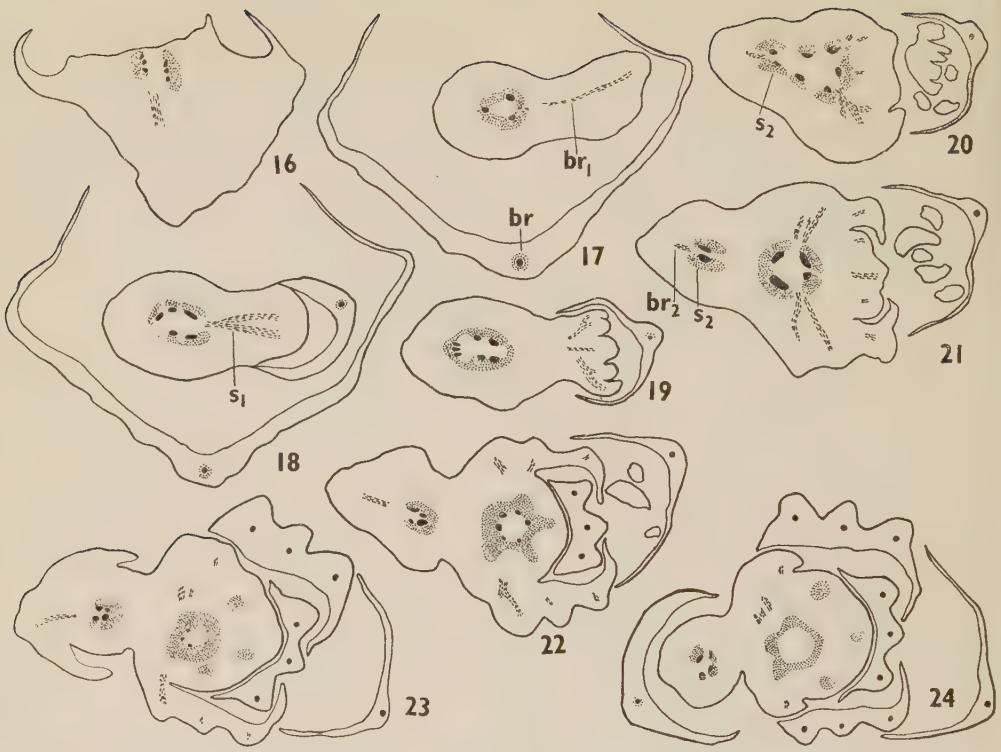
Each sterile flower is morphologically very much similar to the fertile one. It has a pistillode and five staminodes and its bracteoles possess 'scales' in their axils instead of sterile flowers. These 'scales', in contrast to those of *Digera arvensis*, are minute protuberances scarcely visible to the naked eye.

Vascular Anatomy — When the vascular supply to the bract enclosing the flower cluster is to be given out, the main axis shows two vascular bundles (Fig. 16). The first bracteole trace appears at a much higher level (Fig. 17). This is followed by another in the axil of the first bracteole (Fig. 18). The latter soon gives rise to three branches, the outer two of which bifurcate immediately (Fig. 19). At this stage the main stele consists of a ring of vascular bundles. It gives off traces to the second bracteole and its axillant 'scale' on the left side

FIGS. 1-9 — *Digera arvensis*. Fig. 1. Surface view of the 'scale' showing its vascular supply which branches in a dichasial manner. ×15. Figs. 2-9. T.s. flower from base upward (br_1, br_2, bracteole traces; s_1, s_2, traces to 'scales'). ×30.



FIGS. 10-15 — *Digera arvensis*. T.s. flower at various levels. Note the quadristaminate flower in Fig. 15 (a_1-a_5, traces to stamens; d_1, d_2, dorsal traces to gynaecium; p_1-p_5, traces to perianth segments; *v*, ventral trace to ovule). ×30.



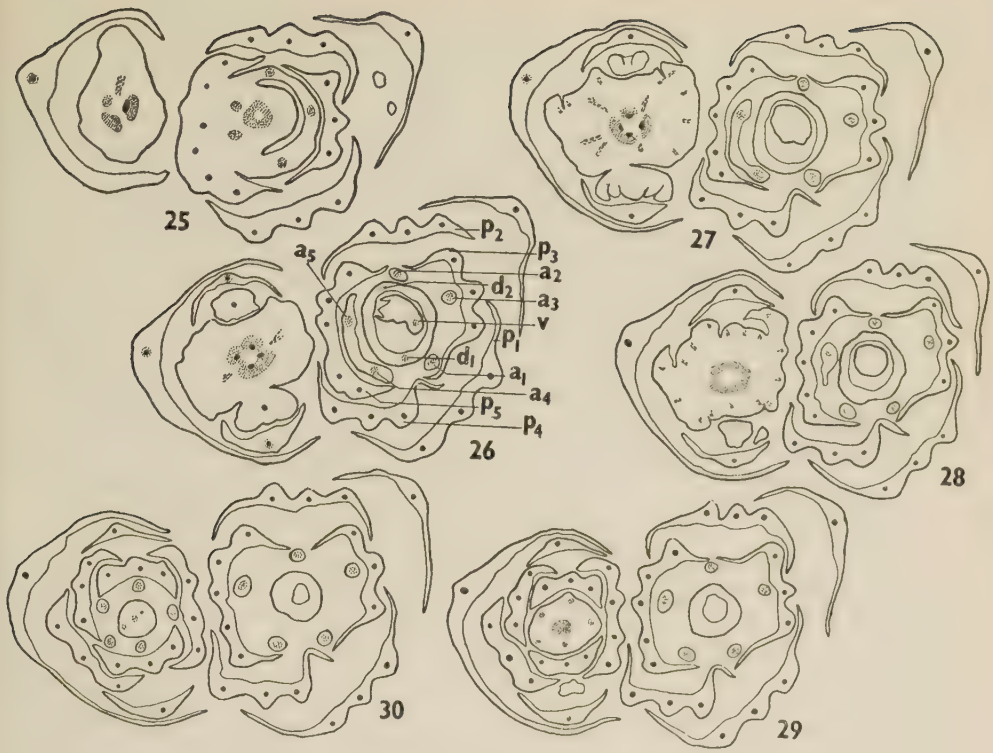
FIGS. 16-24 — *Pupalia lappacea*. T.s. flower cluster from base upward. The large bract is not shown after Fig. 18. Labelling as before. ×40.

(Figs. 20, 21). By this time the central stele has given rise to a number of traces to the perianth segments of the fertile flower. These originate in the order p_1-p_5 (Figs. 20-26). Their median traces come out of independent gaps while the adjacent laterals exhibit a tendency to emerge from a common gap, and occasionally to fuse. The two laterals and the median remain undivided in each perianth segment except in p_5 where each lateral bifurcates so that the segment is 5-nerved (Fig. 26). As each member of the perianth is marked off, the trace to its anteposed stamen protrudes from the central stele. The staminal traces diverge in the order a_1-a_5 and remain unbranched throughout their course. The rest of the stelar tissue organizes itself into three groups. Two of these, at opposite ends, enter the ovary wall as the dorsal bundles, and the third, which is situated centrally, supplies the ovule and is, therefore, the ventral bundle (Fig. 26).

The two bundles given off on the left side (Fig. 21) divide and form a cylinder which behaves exactly like the parent stele in giving out traces to bracteoles, perianth segments, staminodes and carpellodes (Figs. 27-30).

Achyranthes aspera

EXTERNAL MORPHOLOGY — The subsessile flowers of *A. aspera* are arranged in slender, simple or panicled spikes. The two bracteoles are thick, ovate and spinescent. There are five subulate-lanceolate, aristate perianth segments. In the deflexed flowers they are considerably hard and strongly ribbed. There are five stamens whose filaments are fused at the base. The ovary is oblong and a little compressed. The style is filiform and



FIGS. 25-30 — *Pupalia lappacea*. T.s. flower cluster at various levels. ×40.

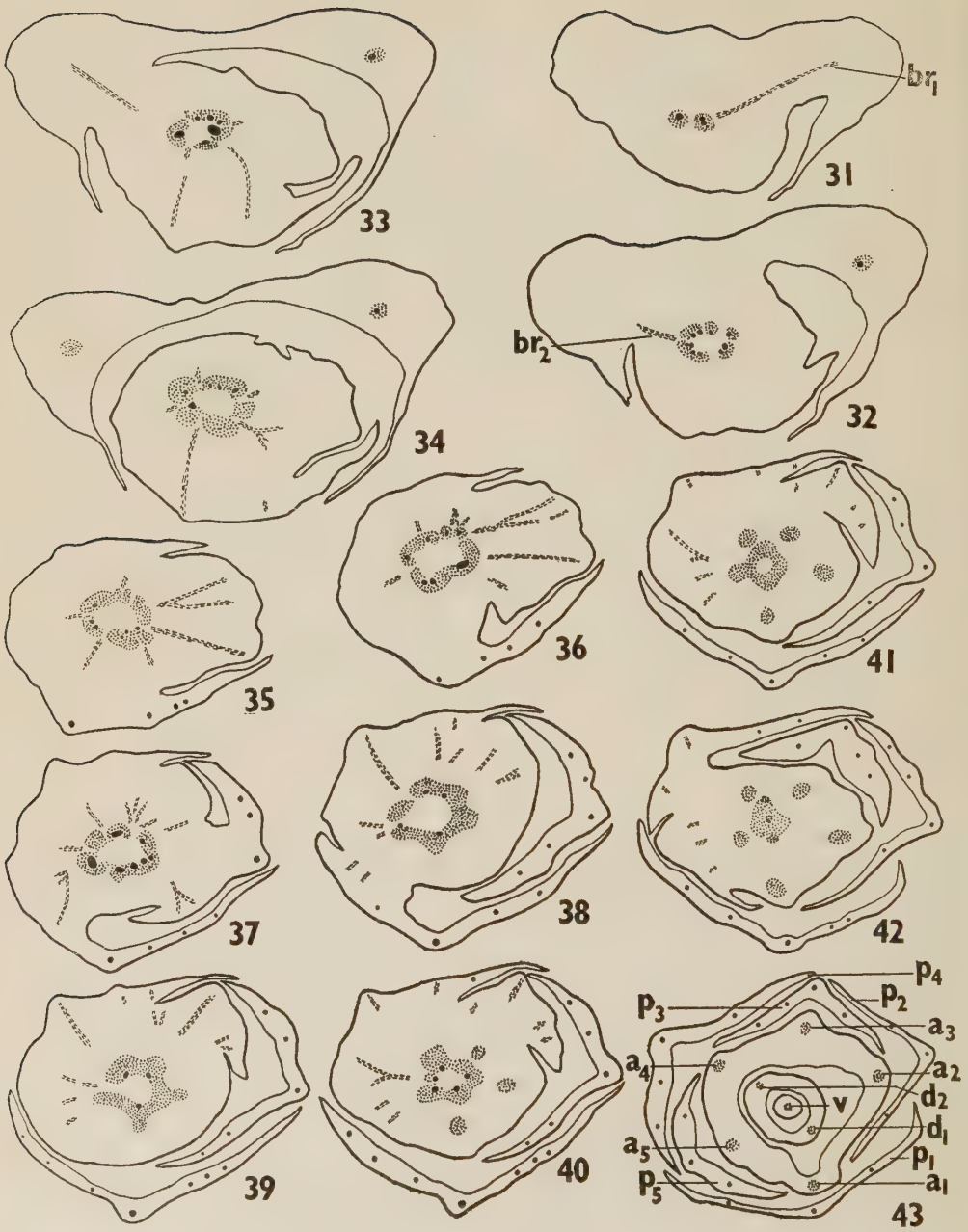
the stigma capitate with a slight indication of its bilobed character. There is a solitary ovule pendulous from a long subbasal funicle.

VASCULAR ANATOMY — When the first bracteole trace is given off, the pedicel of the flower shows two vascular bundles (Fig. 31). These form a ring by the time the second bracteole trace departs (Fig. 32). The traces to the five perianth segments diverge in the order p_1-p_5 (Figs. 33-40, 43). Normally the median traces come out of gaps different from those of the adjacent laterals which exhibit a tendency to fuse. Occasionally, the laterals and the median of the same perianth segment emerge from a common gap (Fig. 37). Higher up in each segment, the laterals usually divide so that the perianth segments may be 3- to 7-veined (Figs. 42, 43).

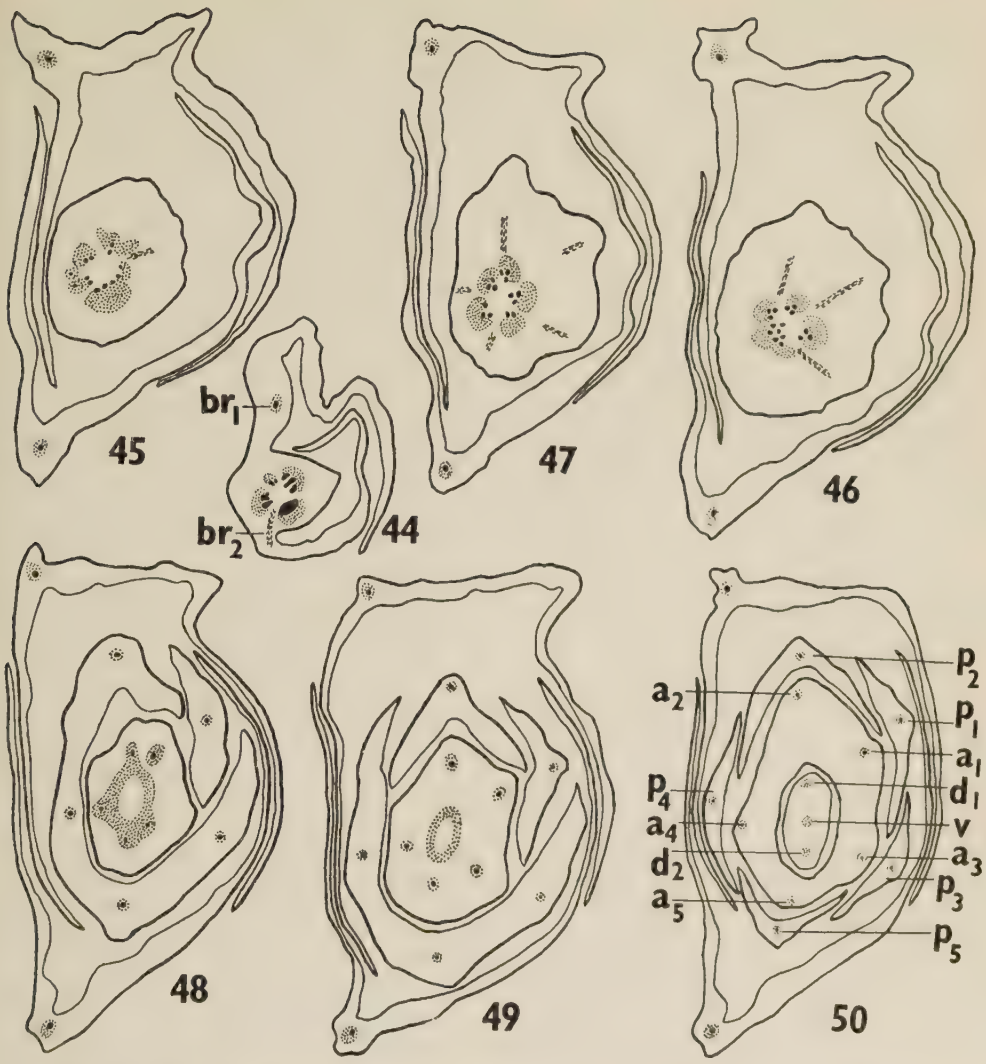
As in the previous two species, the staminal traces arise in the order a_1-a_5 in such a way that a_1 is situated opposite to p_1, a_2 opposite to p_2, and so on (Figs. 39-43). Each of these passes unbranched through the staminal tube. The remaining stelar tissue organizes itself into three groups situated in a straight line. Of these the outer two enter the ovary wall, and the central one supplies the only ovule (Figs. 42, 43).

Gomphrena globosa

EXTERNAL MORPHOLOGY — The pinkish purple flowers of *G. globosa* are grouped in heads subtended by two leafy bracts. Each flower has two large petaloid bracteoles. The perianth consists of five petaloid hard chaffy segments each of which is lanceolate acuminate, and is shorter than the staminal tube. There are five stamens whose filaments are united together into a long tube cleft at the top with one 1-celled anther in each



FIGS. 31-43 — *Achyranthes aspera*. T.s. flower at various levels. The two large bracteoles are not shown in Figs. 35-43. ×35.



FIGS. 44-50 — *Gomphrena globosa*. T.s. flower at various levels. Note the large size of the bracteoles. ×45.

cleft. The ovary is subglobose and unilocular. The style may be long or short ending in two stigmas. There is a solitary ovule pendulous from a long sub-basal funicle.

VASCULAR ANATOMY—The short pedicel shows two vascular bundles each consisting of 2-3 xylem groups. After the departure of the first and second bracteole traces a ring of vascular bundles is formed (Fig. 44) from which the traces to the five perianth segments are given out one after the other (Figs. 45-47, 50). Each perianth segment receives from an independent gap a single trace which remains undivided.

The traces to the five stamens arise spirally in the order a_1-a_5 (Figs. 48-50) and occur opposite the perianth segments. The staminal traces also remain single throughout their course in the long staminal tube.

When the staminal traces have been given off, the remaining vascular tissue arranges itself into three groups. Of these the central one enters the funiculus and is, therefore, the ventral or the placental strand (Fig. 50). The remaining two bundles enter the ovary wall on opposite sides and are to be regarded as the dorsal bundles.

Discussion

The Morphology of the So-called 'Scales'[1] — The nature of the so-called 'scales' within the axils of bracteoles in *Digera arvensis* appears to be somewhat controversial. On the basis of what has been said above we are inclined to think that each 'scale' should be interpreted as a reduced inflorescence or, to be more exact, a reduced dichasium in which the main axis before ending in a flower gives out two branches laterally. These branches behave like the parent axis of the 'scale' (Fig. 1). The vascular bundles in all these branches follow the same course. The condition in *Pupalia lappacea* supports such an interpretation. It will be recalled that in each flower cluster of *P. lappacea* the central flower is always fertile and has on either side a group of sterile flowers. Each group originates in the axil of the bracteole of the fertile flower and has, in addition to the usual floral parts, two 'scales' each situated in the axil of the bracteole of the sterile flower. The 'scales' receive their vascular supply from the sterile flower in much the same manner as does the latter from the fertile one. The condition evidently suggests that the original form of the inflorescence of the flower cluster was a dichasium whose constituent parts now exhibit varying degree of reduction. The apparently single flower of *Digera arvensis*, therefore, appears to be a dichasium of which only the first flower has survived and the two 'arms' have been reduced to just two 'scales'. This indicates that in this particular respect *Pupalia lappacea* is more primitive than *Digera arvensis*.

1. A short account of this has already appeared earlier (Bakshi & Chhajlani, 1953).

This interpretation of the sterile 'scales' appears to be similar to that of Schinz (1934) who regards each of them as a group of sterile flowers and believes that due to reduction certain flowers of the original dichasium got transformed into spines or scales or hairtufts, thus giving rise to convoluted ball-shaped partial inflorescences which can always be resolved into pure dichasia or into dichasia with twisted axes. The species which he mentions in this connection are *Acnida cannabina*, *Pupalia lappacea*, *P. atropurpurea*, *Digera alternifolia* and *Cyathula capitata*. Joshi & Rao (1934) who have studied *Digera arvensis*, however, regard each 'scale' as a reduced flower and consider the apparently simple flower of the species to be "a branch system, a ternate spikelet, bearing one perfect flower at the apex and two reduced flowers laterally".

Morphology of the Perianth Leaves — It will be recalled that the uppermost perianth leaves in *Digera arvensis* usually receive only one vascular bundle while the lowest ones get three traces each. This condition has some morphological significance and can be interpreted in two ways. Either it means that all perianth leaves are derived from stamens which are essentially unitrace structures and that unitrace condition is primitive (cf. Joshi & Rao, 1934) or that unitrace condition for perianth is derived and three-trace condition is primitive (cf. Bakshi, 1952). On account of the following considerations the present authors are inclined to favour the second alternative.

As shown earlier the inner perianth segment on the left (Fig. 10) receives a single trace which soon gives off a lateral branch on its upper side (Fig. 11). Higher up this branch bifurcates (Fig. 14). The condition obviously represents unequal cohesion of the laterals with the median bundle. While the lateral on the side of p_2 has completely fused with the median, the one on the other side has done so only partially as after a time it separates from the median and bifurcates. A similar condition is seen in p_4 with the difference that here the visible lateral does not bifurcate. In p_5 the union of

the laterals and the median is complete and the organ shows only one vascular bundle throughout[2] (Fig. 14). There were, however, a number of flowers which showed only one bundle in each of the three inner perianth segments as has also been reported by Joshi & Rao (1934). This evidently is a condition derived from the original three-trace one. In one flower reduction had gone a step further. Here there were only four perianth segments, two outer and two inner, the latter had one bundle each (Fig. 15). There was no trace of any vascular stubs for the fifth perianth segment or the fifth stamen. Such a condition can, however, be derived by assuming that the traces of p_4 and p_5 approach each other and finally fuse together. Simultaneous with the fusion of these segments their anteposed stamens also unite resulting in a quadristaminate flower. The relation of perianth segments to stamens is, therefore, very intimate — that of protection. This supports the observations of Rendle (1903) and Bakshi (1952) that in typically monochlamydeous flowers with anteposed stamens " the perianth is rather a protective foliar growth of the axis than a modification of the lower whorl of sporophylls ".

The present study supports the views of Bakshi (1952) inasmuch as the different perianth segments of the investigated species show a varying degree of union of their laterals with the median. That this is not improbable is seen from a recent suggestion (Puri, 1951, p. 477) that during specialization of a flower, its typical vascular plan may be modified by cohesion of vascular bundles " either in the basal regions of the organs only or throughout ". Smith (1926) has also shown that in the sepals, of certain Ranunculaceae which are fundamentally three-trace trilacunar organs, reduction has led to the origin of the three traces from a single gap, and eventually to the fusion of such traces into a single strand. The same appears to be true for the Amarantaceae.

As has been shown earlier, *Pupalia lappacea* is more primitive than any other investigated species of the family. The vascular supply to its perianth segments may, therefore, be taken as the starting point for a discussion of the nature of the perianth in the Amarantaceae. Each perianth segment of *P. lappacea* receives three traces arising from independent gaps. Since the flower cluster has undergone considerable reduction there is a tendency amongst the adjacent laterals to fuse and among the laterals and the median of the same perianth segment to originate from a common gap. Further reduction is noticeable in *Digera arvensis* where the traces of only the outer perianth segments originate independently from a common gap while those of the inner segments have fused for part or whole of their course. Obviously in the flowers examined by Joshi & Rao (1934) the inner perianth segments were reduced to the maximum. Since the intermediate stages did not come their way, they interpreted the perianth of the Amarantaceae to have been derived " totally " from the stamens.

The Stamens and the Carpels — According to Joshi & Rao (1934) each staminal trace in *Digera arvensis* bifurcates in the region of the short staminal tube, the branches uniting again as the filaments separate. No such condition could be seen in our material although we examined several series of microtome sections and dissected a number of flowers. The staminal traces, in all species examined here, pass *unbranched* through the staminal tube. In Joshi & Rao's Figs. 11-14 the vascular tissue of the stamen situated opposite to the perianth segment *c* behaves rather peculiarly. In Fig. 11 it shows two bundles which unite in Fig. 12, but divide again in Fig. 13 to fuse for a second time in Fig. 14. If their Fig. 15 (p. 209) is " a reconstruction from a series of transverse sections sketched in text figures 1-14 ", then in the region of the staminal tube the vascular tissue of this stamen should have been represented by a figure of 8.

2. That this series can as well be read in the reverse direction does not seem to be possible. This would be clear from the subsequent discussion where the early history of the Amarantaceae together with the possible reductionary evolution within the family has been traced.

In every case the gynaecium is supplied by three traces all occurring in a straight line. Of these the central one always supplies the ovule. The outer two, the dorsals, may either fade away early (*Psilostachys sericea*, Bakshi, 1952) or may continue a little distance in the ovary wall (*Pupalia lappacea*, *Achyranthes aspera* and *Gomphrena globosa*) or they may reach as high up as the stigma (*Digera arvensis*). The species investigated so far have a bilobed stigma. This condition together with the occurrence of two carpellary dorsals seems to indicate that the gynaecium in the investigated species of the family is essentially bicarpellary.

The Early History of the Amarantaceous Flower — The present study has been significant in explaining the reductionary evolution which has taken place in the inflorescence and the flower of the family Amarantaceae. The original form of the inflorescence appears to have been a raceme of dichasia in which the main axis of each dichasium always ended in a flower and the subsequent growth continued through a bud in the axil of each bracteole. With the onset of reduction, the dichasial axes became shorter and the floral parts reduced. The process continued till there survived in each dichasium only one fertile flower surrounded by the " remains " of others. This is what is seen in *Pupalia lappacea* where the central flower is fertile and has in the axil of its bracteoles a group of sterile flowers. Through further reduction the sterile flowers were lost and the two " arms " of the original dichasium were reduced to mere ' scales '. The ' scales ', as is seen in *Digera arvensis*, retained the dichasial nature of the original inflorescence axes but lost all trace of the flowers borne by them. This reductionary series thus lends support to the present suggestion that each ' scale ' of *D. arvensis* does not represent a single flower as has been supposed by Hooker (1885) and later confirmed by Joshi & Rao (1934), but an entire " arm " of the dichasium. Ultimately, however, the ' scales ' also disappeared. This is evident in *Psilostachys sericea* (Bakshi, 1952), *Achyranthes aspera* and *Gomphrena globosa*. It is now easy to see the final step in reduction through a shortening in length of the raceme itself and to derive the globose heads of *G. globosa*.

In the light of the earlier discussion the morphology of the perianth of the Amarantaceae hardly needs a detailed consideration. As has already been suggested reduction has proceeded from *Pupalia lappacea* to *Digera arvensis* and not vice versa. The perianth of *D. arvensis* has, therefore, been derived from a form with a three-trace supply to its perianth segments. That this is so, is clear from the flowers of *Psilostachys sericea* (Bakshi, 1952) and *Achyranthes aspera* which, on account of the absence of ' scales ', have still retained the three-trace vascular supply even in their inner perianth segments. It may, therefore, be concluded that the perianth of the Amarantaceae has been derived by elaboration of leaf-like organs rather than by sterilization of stamens. Hence the suggestion, that the ancestors of *Digera arvensis* had " naked " flowers (Joshi & Rao, 1934), does not seem to get support from the present study. On the other hand, the family Amarantaceae appears to have originated from an ancient stock whose flowers were very much similar to the fertile flowers of the modern *Pupalia lappacea*. The ancestral flower of the present-day Amarantaceous plants was probably subtended by a bract and two bracteoles, and consisted of five three-veined perianth segments enclosing stamens and a syncarpous gynaecium. All these (bracteoles and perianth segments) were of approximately the same size and shared equally the function of giving adequate protection to the stamens and the carpels. Subsequently this ancestral flower seems to have suffered reduction in probably three different directions:

1. Reduction of the bracteoles but retention of the form of the entire perianth.

2. Reduction of the bracteoles and the inner perianth segments but elaboration of the outer segments.

3. Reduction of both the inner and the outer perianth segments but an elaboration of the bracteoles.

The first of these conditions is seen in *Psilostachys sericea* (Bakshi, 1952), the second in *Digera arvensis*, and the third in *Achyranthes aspera* and *Gomphrena globosa* (the latter being more reduced than *Achyranthes aspera*). It is important to note that the function of protection of the essential whorls never seems to have been neglected during any of these reductionary changes. Thus in *Psilostachys sericea* (Bakshi, 1952) the exposed parts of all the perianth segments are strongly developed; in *Digera arvensis* the outer segments are protective in function; and in *Achyranthes aspera* and *Gomphrena globosa* the function is performed by the bracteoles. Further, with the transfer of the function of protection to either the outer perianth segments or the bracteoles, the reduced inner perianth segments (*Digera arvensis*) or both the inner and the outer segments (*Gomphrena globosa*) became petaloid. Specialization in *G. globosa*, however, went a step further. Here with the aggregation of the entire inflorescence into a globose head, the risks of mortality were minimized. Therefore, the bracteoles too became petaloid. The petaloid colouring in these apetalous flowers is thus due to the gradual transference of the function of protection from the inner perianth segments to the outer, and from these to the bracteoles. The present authors do not agree with Saunders (1933) that the phenomenon of colouring in the perianth parts of monochlamydeous forms can be explained on the basis of the conception of "congenital concrescence". On the contrary, Puri (1951) has suggested that the phenomenon is "a physiological process which may be connected with the flow of certain factors through marginal bundles arising on petal sectors". The present study supports Puri's views in considering the colouring as a physiological process, but it appears doubtful if "marginal bundles arising on petal sectors" have anything to do with the phenomenon, at least in the Amarantaceae. As has been shown earlier, the marginal bundles in the coloured perianth leaves of the Amarantaceous flowers fuse with their respective medians. The question of their causing the petaloid colouring, therefore, does not arise. In the Amarantaceae it is rather their absence through reduction which seems to be correlated with the colouring of the perianth.

Summary

The present study deals with the floral anatomy of *Digera arvensis* Forsk., *Pupalia lappacea* Moq., *Achyranthes aspera* Linn. and *Gomphrena globosa* Linn. In all the four species, the perianth segments are essentially three-trace trilacunar organs. Reduction may, however, lead to the origin of three traces from a single gap, and eventually to the fusion of such traces into a single strand, so that in forms which have undergone maximum reduction, the perianth segments are univeined. Each staminal trace remains single throughout its course. The gynaecium in the investigated species of the family is bicarpellary syncarpous.

The morphological nature of the so-called 'scales' arising in the axils of the bracteoles of *Digera arvensis* has been discussed and it has been tentatively suggested that they probably represent reduced dichasia and not single flowers as has so far been believed.

The nature of the perianth segments has also been discussed in some detail and it has been concluded that they have been derived by elaboration of leaf-like organs rather than by sterilization of stamens. Some remarks have also been made regarding the early history of the family Amarantaceae. It has been suggested, for instance, that the family has arisen from an ancient stock whose inflorescence consisted of a raceme of dichasia, and whose flower was subtended by a bract and two bracteoles and consisted of two essential whorls surrounded by five equally developed three-veined perianth segments. The present-day forms like *Psilostachys sericea*, *Digera arvensis* and *Gomphrena globosa* have been derived from such an ancestral flower through reduction. Further, it is concluded that among the species studied, *Pupalia lappacea* is the least specialized and *Gomphrena globosa* the most reduced.

It is believed that the colouring of the perianth segments in apetalous flowers

is probably due to the gradual transference of the function of protection from the inner perianth segments to the outer and from these to the bracteoles.

The authors are indebted to Dr. V. Puri (Meerut College, Meerut) with whom they had the privilege of discussing the various aspects of the problem. While not claiming his support for the views expressed here, they have great pleasure in recording their sincere thanks to him for the frank discussions, for going through the manuscript and for making valuable suggestions. Thanks are due to Dr. B. N. Mulay, Birla College, Pilani, where this work was carried out, for facilities and encouragement. The senior author is grateful to his friend and student, Mr. Virendra Kumar, for co-operation in the translation of some German passages.

Literature Cited

Bakshi, T. S. 1952. Floral morphology and embryology of *Psilostachys sericea* Hook. f. Phytomorphology **2**: 151-161.

— & Chhajlani, S. L. 1953. The morphology of the scales of *Digera arvensis* Forsk. Curr. Sci. **22**: 313.

Hooker, J. D. 1885. "Flora of British India." **IV.** London.

Joshi, A. C. & Rao, C. V. 1934. A contribution to the anatomy, morphology and cytology of the flower of *Digera arvensis* Forsk. J. Indian Bot. Soc. **13**: 201-236.

Puri, V. 1951. The role of floral anatomy in the solution of morphological problems. Bot. Rev. **17**: 471-533.

Rendle, A. B. 1903. The origin of the perianth in the seed plants. New Phytol. **2**: 66-72.

Saunders, E. R. 1933. The cause of petaloid colouring in apetalous flowers. J. Linn. Soc. Bot. **49**: 199-218.

Schinz, H. 1934. Amaranthaceae. In "Die natürlichen Pflanzenfamilien". **16C.** Leipzig.

Smith, G. H. 1926. Vascular anatomy of Ranalian flowers: I. Ranunculaceae. Bot. Gaz. **82**: 1-29.

SECONDARY GROWTH IN THE LEAVES OF *CHENOPODIUM ALBUM* L. AND *AMARANTHUS GANGETICUS* L. AND THE PARTIAL SHOOT THEORY OF THE LEAF

B. SAMANTARAI & T. KABI

Department of Botany, Ravenshaw College, Cuttack, India

Introduction

Growing an isolated leaf as a separate entity with its own root system generated from the petiole by the application of synthetic hormones is of recent origin. Gregory & Samantarai (1950) studied the histological responses of the petioles of isolated leaves from the time of treatment with hormone till the emergence of roots. Samantarai & Kabi (1953), while growing leaves with hormone induced roots in soil, discovered secondary growth in the petioles. Excepting these two papers, there has been no other work in this line. The present work was undertaken with a view to correlating the secondary growth of the petiole with that of the stem of the same species and to note the histological responses before the emergence of roots. Considerable work has already been done on the anatomy of

stems (Artschwager, 1920; Joshi, 1931, 1937) of plants whose leaves have been used in the present study.

Materials and Methods

Actively growing leaves of *Chenopodium album* and *Amaranthus gangeticus* var. *tristis* L. were treated with an aqueous solution of β-indolyl butyric acid (I.B.A.), *Chenopodium* at a strength of 10 p.p.m. and *Amaranthus* at 5 p.p.m. as these strengths had been found to be optimal for the production of roots. The mode of preparation of the solutions and the method of application are the same as recorded by Gregory and Samantarai (1950). Fifty isolated leaves were treated and an equal number of attached leaves of the same age and size were kept as control. Afterwards the petioles of both the treated and control leaves were fixed in Nawaschin's solution at 2-day intervals till the time of emergence of roots in the treated leaves.

When the roots came out of the petioles, the leaves were planted in soil for the purpose of studying the progress of secondary growth. Materials from the rooted as well as the control leaves were fixed at 7-day intervals, imbedded in paraffin and microtomed. The sections were stained with haematoxylin and safranin.

Observations

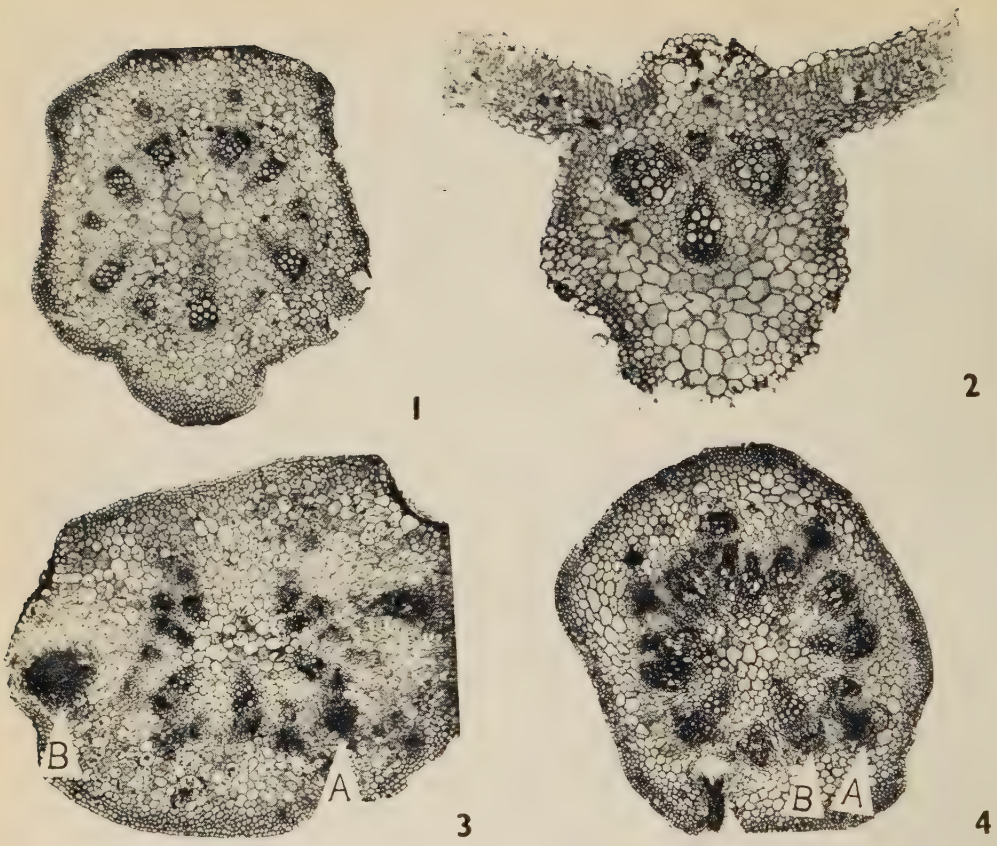
Chenopodium album

Control Leaf — The structure of an attached petiole shows about 13 vascular bundles of which 11 lie in a ring and the other 2 towards the adaxial side (Fig. 1). All the bundles are collateral with endarch xylem. A fascicular cambium is usually absent or occurs only in the form of isolated and inactive cells. No secondary vascular tissues are developed. The cortex consists of parenchymatous cells and the hypodermis is collenchymatous.

At the basal region of the lamina the midrib contains 3 vascular bundles (Fig. 2) arranged in the form of an arc and another small vascular bundle lying toward the upper side of the leaf. As in the petiole, the cambium is inconspicuous and secondary growth is not seen even in the oldest leaf. The mesophyll is normal as in most dorsiventral leaves.

Petiole of the Treated Leaf—Three days after treatment the petioles become swollen. In a week's time root initiation starts in the parenchymatous cells lying outside the phloem as well as in the mid cortex region (Fig. 3). Cells in these regions are found to be in actively dividing condition. Cells of the fascicular cambium also become active (Fig. 4). Secondary growth in the bundles starts much earlier than the organization of the root primordia, which takes place about seven days after treatment. Initiation of all the roots does not take place simultaneously. While some have already been organized and emerged out of the petiole, others are just at the initiation stage. Twelve days after treatment profuse secondary growth is seen in the bundles (Fig. 5) and this phase corresponds to the first phase of secondary growth in the stems of the same species. No interfascicular cambium is formed, so that the petiolar bundles remain separate from one another.

After the leaves are grown in soil, the petioles become thicker. Three weeks after treatment accessory cambia are developed outside the vascular bundles forming almost a ring, except for a very small portion on the adaxial side (Fig. 6). All the cells of these cambia are not uniformly active in the production of secondary tissues. The portions lying just outside the phloems of the vascular bundles are much more active in producing vascular tissue, while at other places a parenchymatous and sclerenchymatous conjunctive tissue is produced. The vascular elements are thus embedded in this conjunctive tissue. Meanwhile, on the adaxial side where the cambium ring was slightly open, a new accessory cambium is developed so as to complete the ring. Conjunctive tissues consisting of parenchyma and sclerenchyma are formed by the activity of this cambium. Due to the differential activity of the accessory cambium and the production of the lignified sclerenchyma and vessels at particular regions and softer parenchymatous tissues at others, the circular

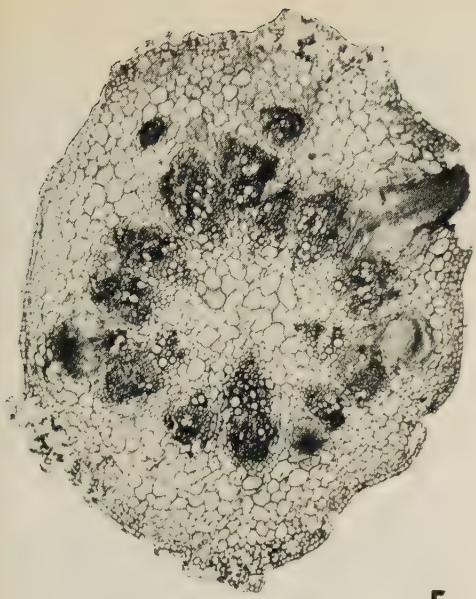


FIGS. 1-4 — *Chenopodium album*. Fig. 1. T.s. petiole showing normal structure. ×34. Fig. 2. T.s. lamina through the midrib showing normal structure. ×70. Fig. 3. T.s. petiole 7 days after treatment (*A*, meristem; *B*, root primordium). ×31. Fig. 4. T.s. petiole, 10 days after treatment (*A*, root primordium; *B*, fascicular cambium). ×29.

contour of the accessory cambium is lost and it becomes more or less wavy and discontinuous at some places. Accessory cambial arcs are formed outside the phloems of the two small adaxial bundles. Their behaviour resembles that of the previously formed cambium outside the initial vascular ring. The time of formation of these arcs synchronizes with that of the formation of the cambium strip, which bridges the discontinuous portion of the cambium band around the ring of vascular bundles.

The final stage is attained about 45 days after the treatment (Fig. 7). The mode of development and the pattern of orientation of these secondary vascular and conjunctive tissues are very similar to those seen in the stems of this species.

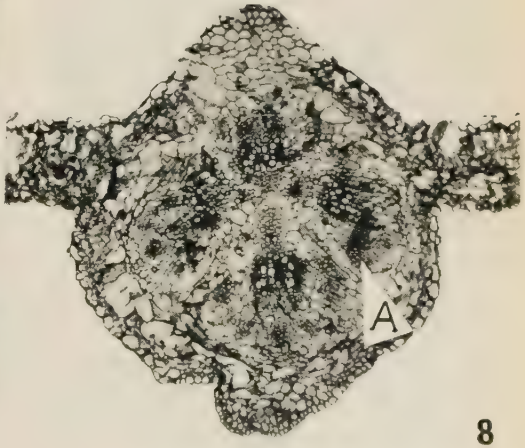
LAMINA OF THE TREATED LEAF — In the rooted leaf, the lamina expands and becomes thicker. The midrib and the main lateral veins also become stouter. A distinct cambial layer is seen in each bundle, which is active and produces secondary vascular tissues. In the midrib as well as in the main lateral veins the development of the accessory cambium is remarkable (Fig. 8). The accessory cambia are developed outside the vascular bundles and produce secondary vascular tissue and conjunctive tissue just as in the petiole and in the stem.



5

6

7



8

FIGS. 5-8 — *Chenopodium album*. Fig. 5. T.s. petiole 12 days after treatment showing secondary growth in bundles; note root primordia at different stages of organization. ×33. Fig. 6. T.s. petiole of rooted leaf (grown in soil) 21 days after treatment showing accessory cambium at *A*. ×35. Fig. 7. T.s. petiole of rooted leaf (grown in soil) 45 days after treatment; note conjunctive tissues at *A*. ×24. Fig. 8. T.s. lamina through the midrib of rooted leaf 45 days after treatment (*A*, secondary xylem developed from accessory cambium in the midrib). ×35.

Amaranthus gangeticus

Control Leaf — The petiole of an attached leaf shows 7 endarch vascular bundles, 5 of which are larger and arranged in the form of an arc and two are smaller and situated on the adaxial side (Fig. 9). A cambium is usually absent but in a few bundles, where it was found, the cells were isolated and inactive. No secondary vascular tissues are produced even in the oldest leaves. The cortex consists of parenchymatous cells and the hypodermis is collenchymatous. The outermost layer is the epidermis.

Treated Leaf — Along with the formation of the meristematic cells which organize the root primordia in the petioles, there is an activation of the fascicular cambia which produce secondary vascular tissues within the bundles. Opposite the vascular bundles a few cells, in the region corresponding to that of the pericycle of the stem, become meristematic. One week after treatment they give rise to root primordia, mostly towards the adaxial side and fewer towards the abaxial and lateral regions. No root primordia are initiated in the cortex as in *Chenopodium*.

Four days after the emergence of the roots the leaves were planted in soil. About a fortnight later the petiole as well as the lamina were found to become appreciably thicker. Secondary growth within the vascular bundles continues due to the activity of the fascicular cambium, and accessory cambial arcs differentiated one outside each vascular bundle excepting the two small adaxial ones. In the beginning only parenchyma cells are cut off by these accessory cambia but later vessels and fibres are also formed. The secondary vascular elements formed from the accessory cambia thus become embedded in parenchymatous conjunctive tissue.

At some places internal to some large vascular bundles a few of the pith cells become meristematic. No secondary vascular tissues or sclerenchyma are produced from this meristem but only parenchymatous cells are produced which later become enlarged (Fig. 11).

As a result of the vigorous activity of the accessory cambia and the formation of secondary xylem from the fascicular cambium, the primary phloem of the initial bundles gets crushed and only the secondary phloem persists. The accessory cambial segments cut off more cells towards the inner side as a result of which there is very little phloem in comparison to the xylem (Fig. 12).

The two small adaxial bundles remain very small. Very little secondary growth occurs in them and this is solely due to the fascicular cambium.

There is no meristematic activity in the cortical cells which simply enlarge. Where the accessory cambia outside two bundles do not fuse, the medullary ray cells but become radially enlarged.

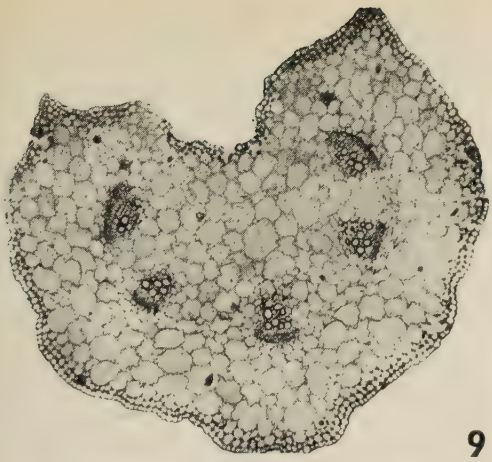
At the final stage, after 2 months, profuse secondary growth is observed in the petiole of the rooted leaf (Fig. 13). The secondary xylem developed from the accessory cambial ring remains embedded in the parenchymatous conjunctive tissue. This induced secondary growth in the petiole of *Amaranthus gangeticus* is essentially similar to that taking place in the stem.

Summary and Conclusion

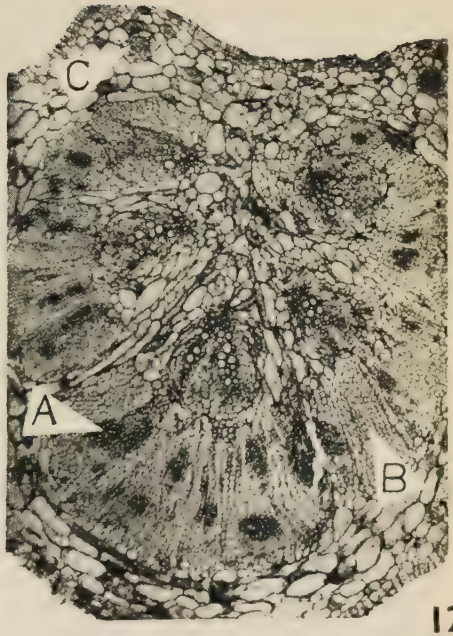
The experimental data reveal three important findings: (a) when detached leaves of *Chenopodium album* and *Amaranthus gangeticus* are treated with 10

—→

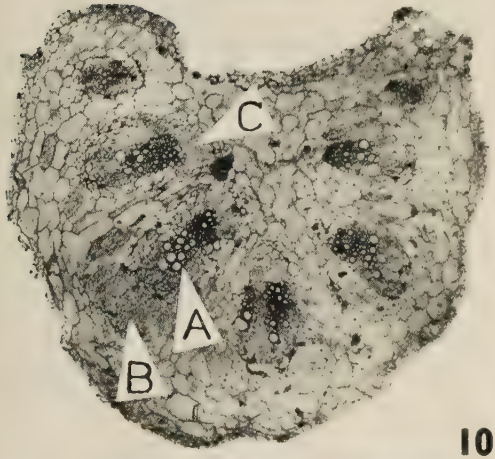
Figs. 9-13 — *Amaranthus gangeticus*. Fig. 9. T.s. petiole showing normal structure. ×44. Fig. 10. T.s. petiole of rooted leaf grown in soil (*A*, secondary xylem developed from the fascicular cambium; *B*, accessory cambium; *C*, meristematic pith cells inside the vascular bundle). ×33. Fig. 11. T.s. petiole of rooted leaf grown in soil (*A*, active accessory cambium cells; *B*, enlarged parenchyma produced from the internal meristem). ×26. Fig. 12. T.s. petiole of rooted leaf 45 days after treatment (*A*, secondary xylem developed from the accessory cambium; *B*, parenchymatous conjunctive tissue; *C*, adaxial bundle). ×25. Fig. 13. Part of t.s. petiole of rooted leaf 2 months after treatment showing final stage of secondary growth (*A*, adaxial bundle). ×29.



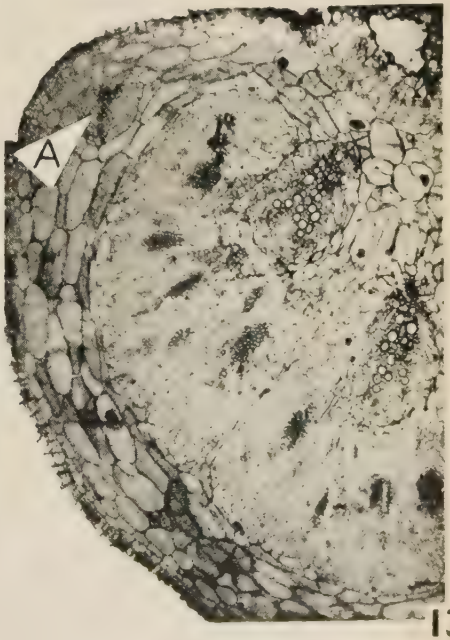
9



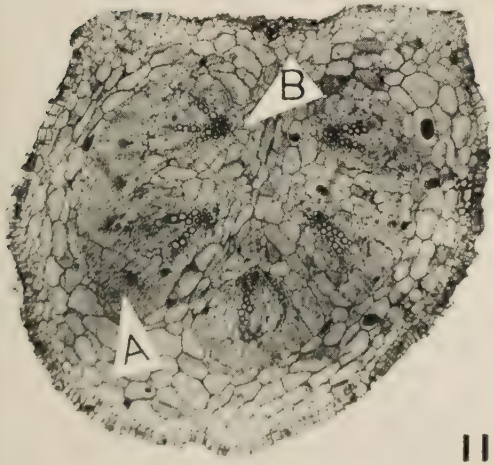
12



10



13



11

FIGS. 9-13.

and 5 p.p.m. of I.B.A., roots arise endogenously as in the stem (cf. Gregory & Samantarai, 1950); (b) simultaneously secondary growth is initiated due to the initiation and activation of fascicular cambium in the vascular bundles. Later an accessory extrafascicular cambium arises and there is further secondary growth; and (c) the leaf with its own root system is capable of living for a much longer time than on the stem and carries on most of its physiological activities as a miniature plant.

The meristematic activity described above is due to the effect of the synthetic hormone with which the leaves are treated. Went and Thimann (1945) have ascribed this meristematic conversion of older cells to the plasticity of the cell wall brought about by hormones. The specific location of these meristems, giving rise to root primordia, outside the vascular bundles is probably connected with the food factor as pointed out by Gregory and Samantarai (1950), and discussed by Burström (1953). The formation of adventitious roots in a region corresponding to the pericycle of the stem is quite significant.

Passing on to the other phase, viz. secondary growth, it may be said that as yet no information is available on the occurrence of secondary growth in the petioles of attached leaves, though Samantarai and Kabi (1953) reported secondary growth in rooted leaves grown in soil. The activation of the intrafascicular cambium and the formation of extrafascicular accessory cambial arcs are induced by hormone applications. Snow (1935) has clearly shown the effect of hormones on cambial activity. The mode of secondary growth in the petioles of *Chenopodium* and *Amaranthus* is very similar to that in the stems of these plants.

Arber (1950) has discussed in detail and produced evidence in favour of the partial shoot theory of the leaf. Our experiments and observations support her views. Not only do the adventitious roots originate endogenously as in the stems, but the mode of secondary growth in the petioles, midrib and veins is essentially the same as in the stems. Moreover, the existence of an isolated leaf with its own root system performing most of its physiological activities not only proves that the leaf is a partial shoot, but also indicates its urge to whole-shoot-hood.

We are grateful to Mrs. A. Arber, Prof. P. Maheshwari, Prof. A. C. Joshi, Dr. I. Banerji and Dr. K. A. Chowdhury for their kind suggestions and encouragement during this investigation. We also thank our colleague Mr. C. M. Bastia for kindly taking the photographs.

Literature Cited

Arber, A. 1950. "The Natural Philosophy of Plant Form." Cambridge.

Artschwager, E. F. 1920. On the anatomy of *Chenopodium album* L. American J. Bot. **7**: 251-260.

Burström, H. 1953. Physiology of root growth. Ann. Rev. Physiol. **4**: 237-251.

Gregory, F. G. & Samantarai, B. 1950. Factors concerned in the rooting responses of isolated leaves. J. Exp. Bot. **1**: 159-193.

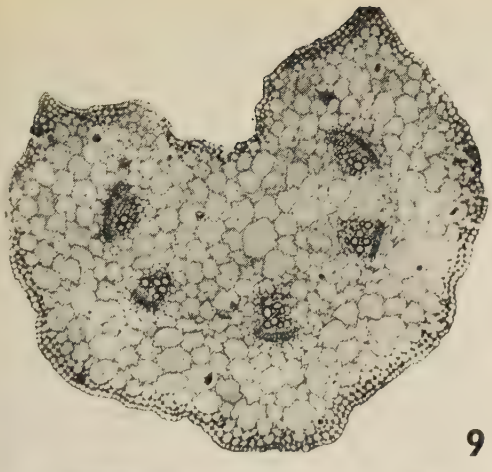
Joshi. A. C. 1931. Contributions to the anatomy of the Chenopodiaceae and Amarantaceae. J. Indian Bot. Soc. **10**: 213-231.

— 1937. Some salient points in the evolution of the secondary vascular cylinder of Amarantaceae and Chenopodiaceae. American J. Bot. **24**: 3-9.

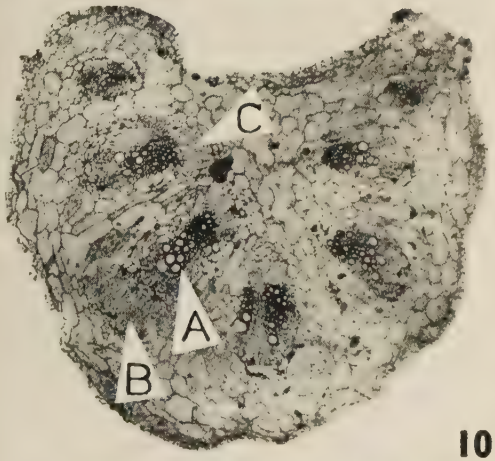
Samantarai, B. & Kabi, T. 1953. Secondary growth in petioles and the partial shoot theory of the leaf. Nature **172**: 37.

Snow, R. 1935. Activation of cambial growth by pure hormones. New Phytol. **34**: 347-360.

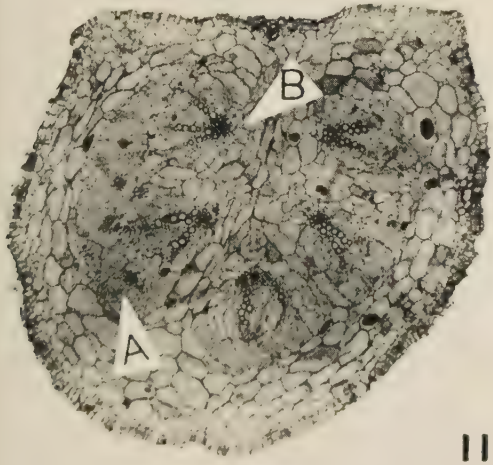
Went, F. W. & Thimann, K. V. 1945. "Phytohormones." New York.



9

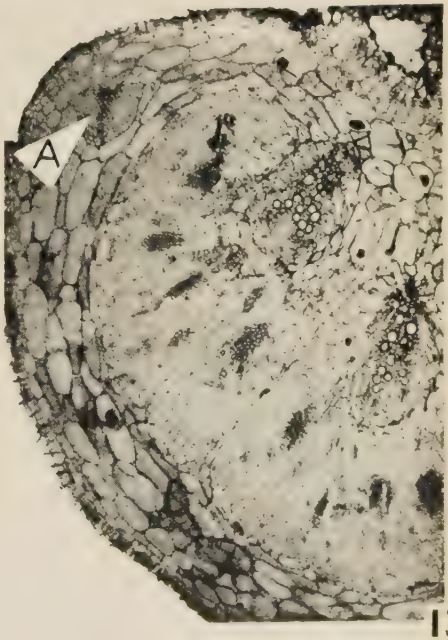


10



11

12



13

FIGS. 9-13.

and 5 p.p.m. of I.B.A., roots arise endogenously as in the stem (cf. Gregory & Samantarai, 1950); (b) simultaneously secondary growth is initiated due to the initiation and activation of fascicular cambium in the vascular bundles. Later an accessory extrafascicular cambium arises and there is further secondary growth; and (c) the leaf with its own root system is capable of living for a much longer time than on the stem and carries on most of its physiological activities as a miniature plant.

The meristematic activity described above is due to the effect of the synthetic hormone with which the leaves are treated. Went and Thimann (1945) have ascribed this meristematic conversion of older cells to the plasticity of the cell wall brought about by hormones. The specific location of these meristems, giving rise to root primordia, outside the vascular bundles is probably connected with the food factor as pointed out by Gregory and Samantarai (1950), and discussed by Burström (1953). The formation of adventitious roots in a region corresponding to the pericycle of the stem is quite significant.

Passing on to the other phase, viz. secondary growth, it may be said that as yet no information is available on the occurrence of secondary growth in the petioles of attached leaves, though Samantarai and Kabi (1953) reported secondary growth in rooted leaves grown in soil. The activation of the intrafascicular cambium and the formation of extrafascicular accessory cambial arcs are induced by hormone applications. Snow (1935) has clearly shown the effect of hormones on cambial activity. The mode of secondary growth in the petioles of *Chenopodium* and *Amaranthus* is very similar to that in the stems of these plants.

Arber (1950) has discussed in detail and produced evidence in favour of the partial shoot theory of the leaf. Our experiments and observations support her views. Not only do the adventitious roots originate endogenously as in the stems, but the mode of secondary growth in the petioles, midrib and veins is essentially the same as in the stems. Moreover, the existence of an isolated leaf with its own root system performing most of its physiological activities not only proves that the leaf is a partial shoot, but also indicates its urge to whole-shoot-hood.

We are grateful to Mrs. A. Arber, Prof. P. Maheshwari, Prof. A. C. Joshi, Dr. I. Banerji and Dr. K. A. Chowdhury for their kind suggestions and encouragement during this investigation. We also thank our colleague Mr. C. M. Bastia for kindly taking the photographs.

Literature Cited

Arber, A. 1950. " The Natural Philosophy of Plant Form." Cambridge.

Artschwager, E. F. 1920. On the anatomy of *Chenopodium album* L. American J. Bot. **7**: 251-260.

Burström, H. 1953. Physiology of root growth. Ann. Rev. Physiol. **4**: 237-251.

Gregory, F. G. & Samantarai, B. 1950. Factors concerned in the rooting responses of isolated leaves. J. Exp. Bot. **1**: 159-193.

Joshi. A. C. 1931. Contributions to the anatomy of the Chenopodiaceae and Amarantaceae. J. Indian Bot. Soc. **10**: 213-231.

— 1937. Some salient points in the evolution of the secondary vascular cylinder of Amarantaceae and Chenopodiaceae. American J. Bot. **24**: 3-9.

Samantarai, B. & Kabi, T. 1953. Secondary growth in petioles and the partial shoot theory of the leaf. Nature **172**: 37.

Snow, R. 1935. Activation of cambial growth by pure hormones. New Phytol. **34**: 347-360.

Went, F. W. & Thimann, K. V. 1945. " Phytohormones." New York.

REVIEW

McLUCKIE, J. & McKEE, H. S. 1954. "Australian and New Zealand Botany." Pp. 758. Associated General Publications, Sydney, Australia. £ (Aust.) 4. 4*s*.

THIS is perhaps the first University textbook from the Australian continent which covers all aspects of botany. One of the authors, Dr. J. McLuckie, is primarily a morphologist and ecologist; the other, Dr. H. S. McKee, is a physiologist. This the reviewer considers to be a particularly happy combination for writing such a text.

The book is divided into three parts of which the first and the best, covering 262 pages, deals with general morphology and physiology. The second part (pp. 263-579) deals with the divisions of the plant kingdom and life-histories of the various representatives of Cryptogams and Phanerogams. The third part (pp. 580-758) covers the remaining topics like systematic botany, heredity, palaeobotany, and ecology and plant geography. Three special chapters are devoted to the floras of Australia and New Zealand and to the development of Australian botany as a whole.

There is so much that is extremely good and commendable in the book that it would certainly be of considerable interest to teachers and students of botany even outside Australia. There are only a few criticisms the reviewer wants to make. On page 128 in the diagrams showing secondary growth in roots in Fig. 157D the cortex is still shown intact, although in a root with such advanced secondary growth it is likely to be replaced by cork. This is a common mistake with many authors and needs correction. On page 258 the enzymes, maltase and sucrase are listed under glycosidases although they are really carbohydrases. On page 411 the pits on the tracheids of *Pinus* should have been shown on the radial instead of the tangential walls. A more serious error occurs on page 438 in connection with the account of the development of the embryo sac of *Lilium*. The authors seem to be unaware of the work of Bambacioni and Cooper who showed that here the spindles of the three megaspore nuclei at the chalazal end fuse to give rise to two triploid nuclei so that at the mature embryo sac stage the four micropylar nuclei are haploid and the chalazal nuclei are triploid. On page 440 it is stated that in *Casuarina* the pollen tube enters the embryo sac through its chalazal end. This is incorrect because while the pollen tube enters the ovule through the chalaza, it has now been shown that it grows over the whole length of the embryo sac and enters it, as usual, through the end containing the egg apparatus.

The paper, printing and binding are of a high standard and a considerable amount of useful matter is compressed inside a single volume.

P. MAHESHWARI

SUGGESTIONS TO AUTHORS

1. Unless confusion would otherwise arise, the author's name should appear as initials (but female authors may use one given name in full) and surnames only, without titles or suffixes. The name and address of the laboratory where the work was performed should be noted immediately below author's name. Any necessary descriptive material regarding the author, e.g. Senior Fellow, National Institute of Sciences, India, or details of financial support, should appear as a footnote on the first page, or preferably, in the acknowledgements at the end of the paper.

2. Articles will be received only from members of the International Society of Plant Morphologists and may be written in English, French or German. Although no definite limit can be laid down, papers should not ordinarily exceed 20 printed pages. While it is recognized that full detail is essential in many cases, a good many articles can be shortened by changes in wording, by avoiding a description of what is already well known, and by elimination of repetition.

3. Authors are requested to examine this copy of Phytomorphology to note the general organization, position of headings, punctuation and abbreviations in order to bring the script into conformity with the general style of the journal. Footnotes should be numbered consecutively as 1, 2, 3, etc.

4. In many manuscripts sent to the editors, footnotes, bibliographies, descriptions of figures, etc., are typed single spaced. Since such material requires as much editorial marking as the rest, authors are urged to use double spacing throughout.

5. Literature citations are to be typed double spaced like rest of the manuscript. Give complete citation: author, year, title, name of journal, volume number and inclusive pages. In abbreviating names of journals follow " World List of Scientific Periodicals ", or the " U.S. Department of Agriculture list of abbreviations ", or the list given in Biological Abstracts. The words ' Indian ' and ' Indiana ' should not be abbreviated. A sample citation is given below:

STOKEY, A. G. 1948. The gametophyte of *Actiniopteris australis*. J. Indian Bot. Soc. **27** : 40-49.

6. The patterns of headings should be as simple as possible and not more than three classes are to be used. Headings of the first class should be underscored with a bold wavy line, of the second with two parallel straight lines and of the third with a single straight line.

7. Drawings should be made with pen and undiluted India ink for reproduction by the zinc etching process. Group as many drawings as possible and mount them close together on heavy white cardboard for reduction to the width of a single column (6 cm.) or to the full width of the page (13 cm.). A part or all of the length of the page (20 cm.) may be utilized but it would be helpful if some space is left at the bottom to permit insertion of legend below the figures. It is not possible to accept loose figures or figures so mounted as to leave large unused spaces between them. Author's name and figure number must be written on the back of each mount. Do not affix legends to the figures but type them double spaced on a separate sheet. Photographic prints should be glossy with strong contrasts. Line drawings will be preferred, however, both because of the lower cost of reproduction and their relative clearness. When submitting manuscript, give the original magnifications of the illustrations. These will be revised later in accordance with the reduction to which the figures are subjected in reproduction. Alternatively give a dimensional scale in microns or mm. for each figure or for each group of figures differing in magnification.

The thickness of the lines and dots should be planned with the desired reduction in mind; it is also important to calculate the requisite spaces between lines and dots.

Illustrations (including tables and graphs) should not exceed 20 per cent of the text; authors of more copiously illustrated articles may be asked to pay for the excess.

8. Latin names of plants should be used in all cases in preference to common names, and when a plant is comparatively little known, the name of the family should be given in brackets. Underline both generic and specific names throughout the manuscript. Specific names should always be written with a small letter.

9. Place all acknowledgements at the end of the paper just after " Summary " and before " Literature Cited ".

10. It is extremely important that galley proofs are promptly returned to the editor. Authors will be billed at cost for alterations in proofs other than corrections of printer's errors. It is useful to write across top of title page of manuscript directions for mailing of proof, thus: Mail proof to (author's name and vacation address, if any). If authors wish to have original illustrations returned subsequent to publication, the editor should be so informed at the time proof is returned.

11. Order for reprints should be sent to editor with corrected galley proof. Type is destroyed immediately after printing; hence it becomes impossible to supply reprints after publication of papers. Thirty reprints are supplied free to the author.

LA CELLULE

JOURNAL OF ORIGINAL INVESTIGATIONS IN CYTOLOGY AND HISTOLOGY

Founded by

J. B. CARNOY IN 1884

Edited by

PROFESSORS P. DEBAISIEUX (ZOOLOGY) AND P. MARTENS (BOTANY AND CYTOLOGY), LOUVAIN, BELGIUM

Three numbers constitute a volume — generally annual — of 400 to 450 pages quarto with numerous double-page plates and text-figures. Volume 56 (1953-54) in course of publication.

Subscription price per volume: frs b. 550 (= $ 11.00) post paid. Address the Editor: C. UYSTPRUYST, LIBRAIRIE UNIVERSITAIRE, RUE DE LA MONNAIE, LOUVAIN, BELGIUM.

General indexes for volumes 1 to 40 (1885-1931) and author index for volumes 41-50 (1932-1946): 110 pages quarto; price separately: frs b. 100 (= $ 2.00).

For all other communications (editorial, business, exchange, etc.), address: PROFESSOR P. MARTENS, LOUVAIN, BELGIUM, RUE MARIE-THÉRÈSE 23, or INSTITUT CARNOY, 9, RUE DU MANÈGE.

PLANT BREEDING ABSTRACTS

PLANT BREEDING ABSTRACTS is a quarterly journal containing abstracts of current literature throughout the world. All publications having a direct or indirect bearing on the breeding of economic plants are mentioned, the fields covered including genetics, cytology, evolution, practical improvement by selection and by more modern methods such as induced mutation and polyploidy, the use of hybrid vigour in raising yields, and the application of interspecific crosses to utilize the valuable genes of wild and indigenous floras. Not only the commoner crop plants are considered but also vegetables, temperate and tropical industrial plants and fruits, and even forest trees. A large section is also devoted to the genetics of micro-organisms such as fungi, bacteria and virus, which are of interest both theoretically as material for study of the basic principles of heredity, and practically, for producing improved strains for brewing and other industrial purposes, and also for building up disease-resisting forms of agricultural plants.

A special feature of PLANT BREEDING ABSTRACTS is that works published in the more unfamiliar languages are abstracted extensively, so that readers are able, for instance, to follow the important contributions of Japanese investigators to genetical and cytological theory, and to the improvement of special crops such as rice; and more controversial issues such as the recent genetics controversy in the Soviet Union. During the last two years abstracts, in English, of articles written in 31 different languages have been published.

Readers are kept up to date concerning recent developments by two further sections: the Book Reviews, which present objective criticisms of all the more important books and monographs published on the subject, and New Journals, in which readers are informed promptly of the appearance of any new periodical publication in the above-mentioned fields, with indications of the nature of its contents and how to procure it.

An author and classified subject index is included in the subscription price for each volume.

PLANT BREEDING ABSTRACTS is produced and edited by the Commonwealth Bureau of Plant Breeding and Genetics, School of Agriculture, Cambridge, England, on behalf of the Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Nr. Slough, Bucks. Subscription rates are 60s. per volume (with subject index), less 20 per cent to subscribers in the British Commonwealth (other than recognized booksellers) who send their subscriptions direct. Orders may be placed through booksellers or sent to

COMMONWEALTH AGRICULTURAL BUREAUX
CENTRAL SALES BRANCH
FARNHAM HOUSE, FARNHAM ROYAL
NR. SLOUGH, BUCKS.